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An ultrasonic Bragg scattering technique for
the quantitative characterization of marbling in beef

by

Daniel John Haumschild

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INTRODUCTION

Quality grading, even when performed by qualified personnel, is a source of concern for most livestock producers. Subjective evaluation can be influenced by extraneous factors. Hopefully, with the use of ultrasound, objective criteria necessary for consistent grading practices may be established.

Ultrasound systems operate much the same as sonar or radar techniques. Following a transmitted signal, echoes are received from various obstructions or discontinuities in the beam path. Ultrasound became an established technique in the medical field shortly after its introduction in the early 1950s. Agricultural personnel quickly discovered parallel uses. Since the early 1960s, ultrasonic instruments have been used for measurement of backfat layers in swine and more recently for pregnancy checking in sheep, cattle, swine and horses. This same technique is also available for loin measurements in these species. The noninvasive ultrasound method is more desirable than the potentially harmful metal probes previously used for backfat measurements or the exhaustive and possibly damaging palpation technique currently used to detect pregnancy in large farm animals.

The modification, then, of existing ultrasound units for a different type of tissue identification, in this case the marbling characteristics of beef, shows promising results. The mechanics of detecting the degree of marbling (intramuscular fat deposits) is somewhat different than that of determining backfat thickness or loin depth. What changes is that portion of the ultrasound signal that is analyzed and the electronic methods for signal processing. A system such as this would be free of human judgmental errors, providing a standardization that the beef producer industry could easily adopt.

It is in discriminating those cases near the boarderline between any grades that objective methods are most needed. Even skilled observers lack consistent visual assessment from day to day and week to week.

Due to production costs, primarily that of cattle feed, it is desirable to have any given animal make the lowest rank of the choice grade. On a live weight basis, the producer's highest return is at that level of finish. Higher levels do not significantly increase the value but result in proportionally less profit per animal. A larger turnover of animals finished to that point means better financial return for the producer.

Obviously, once the animal has been slaughtered, it is impossible to do anything to improve the quality. It is desirable to have some in vivo method to determine grade category. If the grade does not quite fit the desired category, it is not too late to feed the animal for a short period of time and significantly increase the return on investment. The purpose, then, of developing this ultrasonic instrument is twofold: to allow for accurate and consistent grading of carcasses and to lay the groundwork for development of an instrument which would allow the producer to measure the in vivo degree of marbling.

The ultrasonic test that was used in this project is similar to an analytical crystal characterization procedure known in X-ray crystallography as Bragg scattering. In regard to ultrasound, the echoes from the meat/fat interfaces of various spacings, such as is found in different grades of meat, will interact differently depending on the angle and the frequency of the sound beam. At specific angles, depending on the distance between the reflecting surfaces, the ultrasound echoes interact constructively or destructively with the result recorded as a strong or a weak echo following processing by the instrument. If any given grade of meat is of an ordered or semi-ordered marbling structure, the ultrasound system would be successful.

After a chemical extraction to determine the absolute fat content, multivariate analysis was used to statistically derive a relationship with the ultrasonic record and the fat content. By Fourier analysis, the raw data were first transformed to separate spatial frequency components to provide suitable numbers for the statistical analysis. The spatial frequency as defined here is as opposed to that frequency generally regarded as a time-based function. In this experiment, spatial frequency refers to those frequencies of an ultrasonic signature derived as an angle-based function. The procedure was completed with double as well as single transducer systems.

LITERATURE REVIEW

Historic Review

The use of sound as a tool for the detection of flaws has been known for centuries. In early times, owners of fine crystal glassware found that those pieces of glassware with minute cracks either invisible or barely visible to the naked eye could be detected by listening to the ringing of the glass after lightly tapping it with a rod.

Wells (1969) states that the history of pulse-echo ultrasonic flaw detection is somewhat uncertain. It is known that an ultrasonic depth sounder had been devised prior to 1920. A similar method of analysis for proposing flaws in metals appeared about 15 years later. The earliest publication mentioned by Wells is that of Firestone (1945). Apparently, wartime secrecy did not allow for publications much earlier than this one.

It was not long after the war that the importance of ultrasound in medical diagnosis was realized. Wild (1950) demonstrated its use for measurement of tissue thicknesses and changes of tissue density. French et al. (1950) found that the detection of cerebral tumors was possible under certain conditions. Ludwig and Struthers (1950) had similar success in detecting gallstones. Later publications illustrated the application of ultrasound for visualization or detection of various soft tissues (Wild and Ried, 1952; Howry and Bliss, 1952).

These and several other publications during this same period of time set the stage for a vast new field of medical diagnostics. The ultrasonic medical developments were soon followed by parallel developments in the agricultural industry. A summary of the early agricultural uses and what followed is discussed by Stouffer and Westervelt (1977). As ultrasonic testing has been commonly accepted for only about 15 years, it is still considered to be in the early stages of development.

Related Topics

Echoes from tissues are generally believed to be a function of the scattering process occurring within that tissue and as such are likely to be dependent on the arrangement of scatterers within that tissue. A Bragg scattering approach was tried in an attempt to assess the configuration of scatterers. This was done by changing the orientation of the scatterers relative to the interrogating beam and recording the change in the configuration of the backscattered echoes. Using a 1.0 mHz transducer and a time gated echo, tissue samples were rotated within the transducer beam. The results indicated that the characteristic spacing of beef muscle fasiculae were consistent with theory (Nicholas and Hill, 1975; Nicholas, 1976; Hill, 1976).

When analyzing various tissue types such as liver, brain and spleen along with pathologically varying samples of liver, it was reported that via Bragg scattering, qualitative differences could be characterized, but quantitative characterization could not easily be done. Nicholas and Hill (1975) reported a shift in the reflected power spectra of a Fourier transform of a normal versus a cancerous liver. This indicated that structural differences of the tissues must cause the variation in back-scattered amplitude, as this was the pathologically varying parameter between the normal and abnormal specimens. A technique using B-scan also resulted in similar qualitative results (Huggins and Phelps, 1976).

By maintaining a fixed angle and varying the frequency, it was found that specific types of similar tissues could be distinguished (Chivers and Hill, 1975). Apparent echo frequency (cycles/degree) differences were observed with pig and human liver samples.

By using a 2.25 mHz signal frequency, which optimizes ultrasonic resolution and penetration depth, it may be possible to detect the percent lipid in a fatty liver with a high confidence level (Freese and Lyons, 1979). Although the correlation was not good for normal livers, those livers with abnormally high lipid content appeared to have a backscatter coefficient that was highly linearly dependent ($r = 0.98$) to the percent lipid. For this analysis, frequencies below 2 mHz did not yield quantitative results.

Lele et al. (1976) used angle scanning to detect periodicity differences in several tissue types. Among those samples checked were calf cardiac and skeletal (longitudinal and perpendicular cuts) muscle and calf and pig liver. In testing for frequency dependence versus average periodicity, the slope and intercept values of these tissues differed significantly when statistically analyzed. When comparing previously frozen to fresh and to fixed samples of tissue, these periodicity values were found not to vary statistically although a trend toward lowering of the angular values was observed. Frequency dependent acoustical attenuation or absorption did not occur.

It has been found that normal and abnormal lung surfaces could be characterized by ultrasound (Sagar et al., 1978). At a particular frequency, destructive interference produced a signature in normal lungs notably different from those lungs inflicted with pulmonary emboli or pulmonary diseases. That particular frequency of 5.5 mHz agrees with the theory of Bragg scattering in that one-half of the ultrasonic wavelength at this frequency equals the depth of tissue between adjacent alveolar sacs of normal lungs. Due to the fact that the destructive interference at this frequency did not occur with diseased lungs, it was assumed that air in the alveoli was displaced secondary to either pulmonary emboli or to other infiltrative pulmonary disease.

Experimental observation of swept-frequency acoustic diffraction of nylon arrays with various spacings agreed well with theoretical predictions. As reported by Gramiak et al. (1976), Fourier transforms of the data indicated reliability within 5% of measured values. Angle scanning at a fixed frequency of a random grating indicated that scattered ultrasonic pressure was correct for first-order diffraction by a regular array, with spacing equal to the average spacing of the random array.

The application of this concept to biological samples of pig and human liver demonstrated differences possibly due to pig liver having collagen arranged in a smaller more uniform matrix when compared with human liver. It remains to be determined whether the observed peaks were due to randomly spaced scatterers or to a few large single scattering objects. It was also concluded that care must be taken to develop an alignment procedure for tissue examination so that spacing measurements of tissue features could be properly interpreted (Gramiak et al., 1976; Waag et al., 1979).

With a frequency range of 2.3 to 4.8 mHz, Freese and Hamid (1974) found a correlation of backscatter to lipid content of whole fish. In the absence of gas bubbles and other large coherent scatterers, the measurements revealed a strong dependence of the backscatter on the total lipid content. The total lipid content could be determined to within ± 0.4% over a 2% to 5.4% range.

Tissue testing for ultrasonic attenuation, as a function of time after excision, has shown no appreciable change for up to 5 days. Bovine brain, spleen and liver and porcine spleen and liver were stored at room temperature during this time period. Backscatter amplitude decreased 10 dB during this interval (Bamber et al., 1977).

The explanation for this is given by the belief that absorption of ultrasound is due to macromolecular structures, particularly proteins (Pauly and Schwan, 1971). At 5 days, the in vivo specimen proteins may not be degraded to much lower molecular weights than are present in vivo (Dunn et al., 1969). Conversely, backscattering is believed to occur at structural levels commensurate with the ultrasonic wavelength (Nicholas and Hill, 1975).

With no attempt at quantitative analysis, Gammel (1981) found that a qualitative difference could be observed when ultrasonic backscatter was recorded from beef round. The backscatter is attributed to the marbling characteristics of the meat, however no allowance appeared to be made for the various muscles that would be encountered by the ultrasonic beam.

BACKGROUND INFORMATION

Basic Physics of Ultrasound

Transducer concepts

Pulse-echo ultrasonic examination operates much the same as a voice echo from a canyon wall. The sound is reflected back to the source and is interpreted by the ear as an echo. Only a small portion of the sound wave is reflected back as an echo. Most of the energy continues on into and through the interface (wall).

Ultrasound is that portion of sound that is of too high a frequency to be detected by the human ear. Frequencies above 20,000 Hz are generally considered as ultrasonic. For optimum resolution in diagnostic work, most frequencies are between 1 and 10 mHz.

Ultrasonic instruments generate this signal with a specialized crystal located in the transducer. A piezoelectric crystal is a material that will oscillate or ring about its normal position when excited by a voltage pulse. Materials commonly used for this are quartz, lithium sulfate, barium titanate and lead zirconium titanate. The crystal thickness determines the operating frequency of the transducer. When the crystal thickness is cut to one half of the desired wavelength, a resonant situation will exist which produces a peak output at the operating frequency. When the transducer is coupled to another medium such as tissue, the resonant energy will be delivered to that tissue. Depending on the shock excitation and the damping of the crystal, a continuous wave (CW) or pulsed wave (PW) technique is obtained (Figure 1).

Wave propagation in tissue

Sound waves travel through a medium via periodic disturbances or displacements of particles within the media (vibrations). When the vibration occurs in the direction of sound propagation, the motion is called a longitudinal wave. Motion perpendicular to sound propagation is called a transverse wave. All materials can support longitudinal waves while only solids can support transverse waves. With the exception of bone, body tissues behave as a fluid. Since only soft tissues are of concern, all discussion of sound waves will relate to longitudinal waves only.

Figure 2 shows propagation of a longitudinal wave through a medium. Local pressure within the medium is directly proportional to particle spacing within the structure. As the medium does not vibrate in perfect synchrony, the spacing between particles is not constant. While the sound wave travels through the medium, the interparticle spacing changes so that low density particle areas will become high density and vice versa. At the same time, the distances between high and low densities remain constant and regular. The net effect is the appearance of propagation of sound through the tissue (Goldberg et al., 1975).

Figure 3 is an illustration of what happens to a burst of sound traveling through two mediums. At the interface, a small portion of the

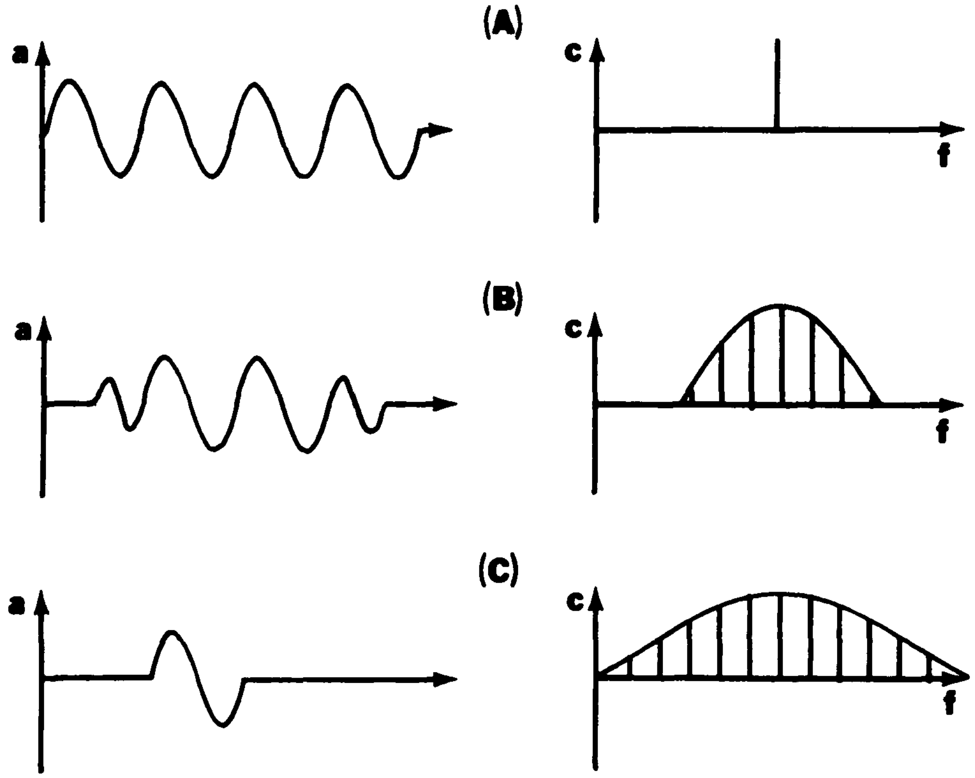


Figure 1. Frequency characteristics of pulsed waveforms and typical associated frequency spectrums. Continuous wave, CW (A), pulsed wave, PW (B), and highly damped (C) waveforms are shown (Rose and Goldberg, 1979)

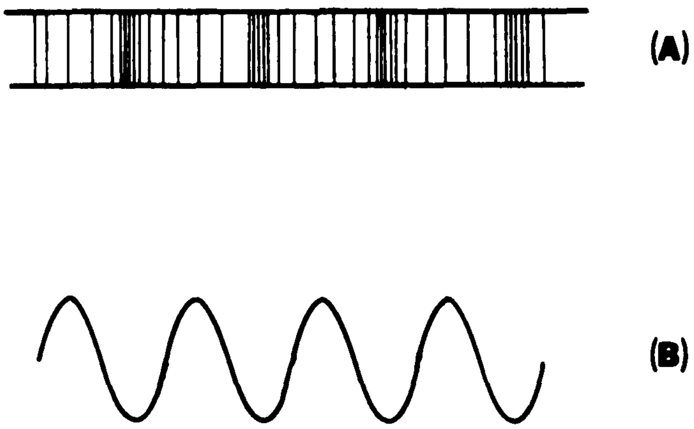


Figure 2. Propagation of a longitudinal sound wave (A), and the corresponding pressure wave (B)

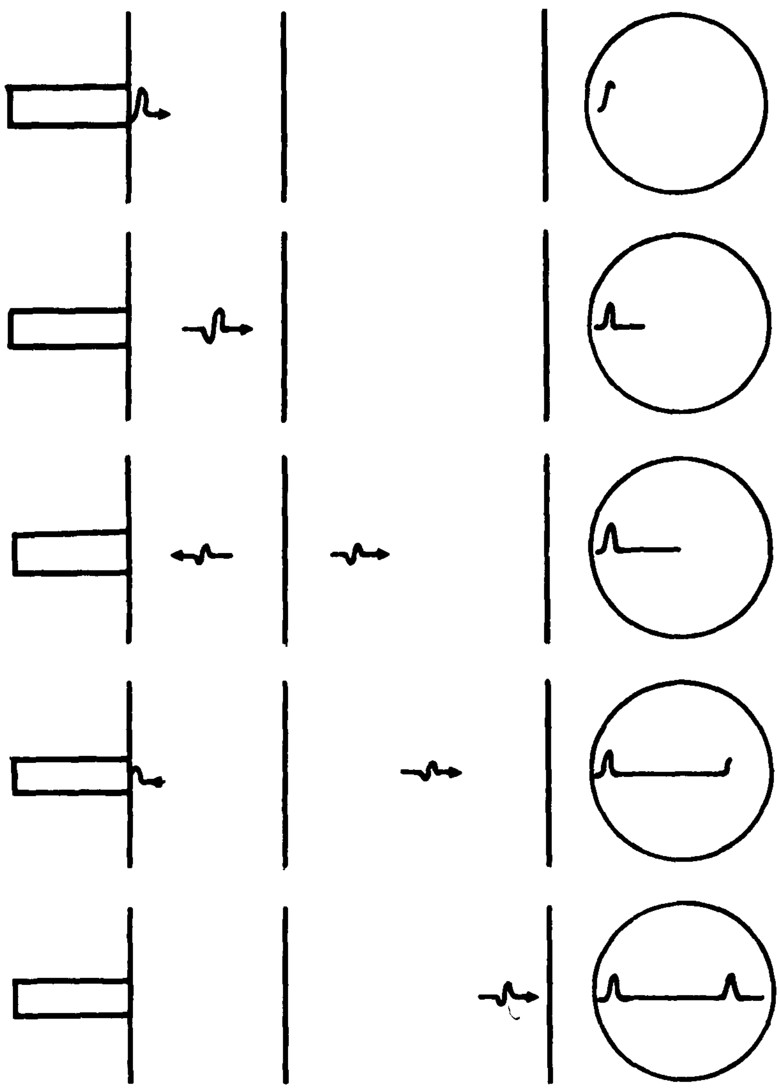


Figure 3. Principles of the A-mode recording technique. The picture sequence from top to bottom shows a sound pulse traveling through two adjoining mediums. The display to the right represents the recording obtained. The two spikes represent the transducer-medium boundary and the boundary between the two mediums, respectively (Fink, 1979)

wave is reflected back toward the transducer while much of the energy continues on through the interface (Wells, 1969). With ultrasound, the angle of incidence equals the angle of reflection from smooth surfaces, in the same fashion as that of light reflecting off a mirrored surface. This is referred to as specular reflection. It is apparent from this that the echo amplitude is greatest when the incident beam is perpendicular to the reflecting surface. At a deviation angle of greater than 2°, the echo amplitude is reduced enough to make detection by the sending transducer virtually impossible (Rose and Goldberg, 1979). In order to detect echoes where the incident wave is more than a few degrees off normal, it is necessary to have a receiver separate from the transmitter. For focused transducers, off normal angulation may not as drastically affect these results (Lele et al., 1976).

Resolution

The ability to distinguish closely reflecting structures which lie in separate planes parallel to the transducer face is known as axial resolution (Figure 4). When the ultrasonic pulse length is long, as in the CW mode, axial resolution is considered to be very poor. In most pulse echo systems, the pulse length is kept as short as possible to improve axial resolution. The trade-off comes in the frequency spectrum (Figure 1) present in short versus long pulses and the total energy available with either one. Since short bursts have less energy than long bursts, the idea is to have enough energy available to be able to detect the echoes. With sufficient energy, it is necessary, then, to have the

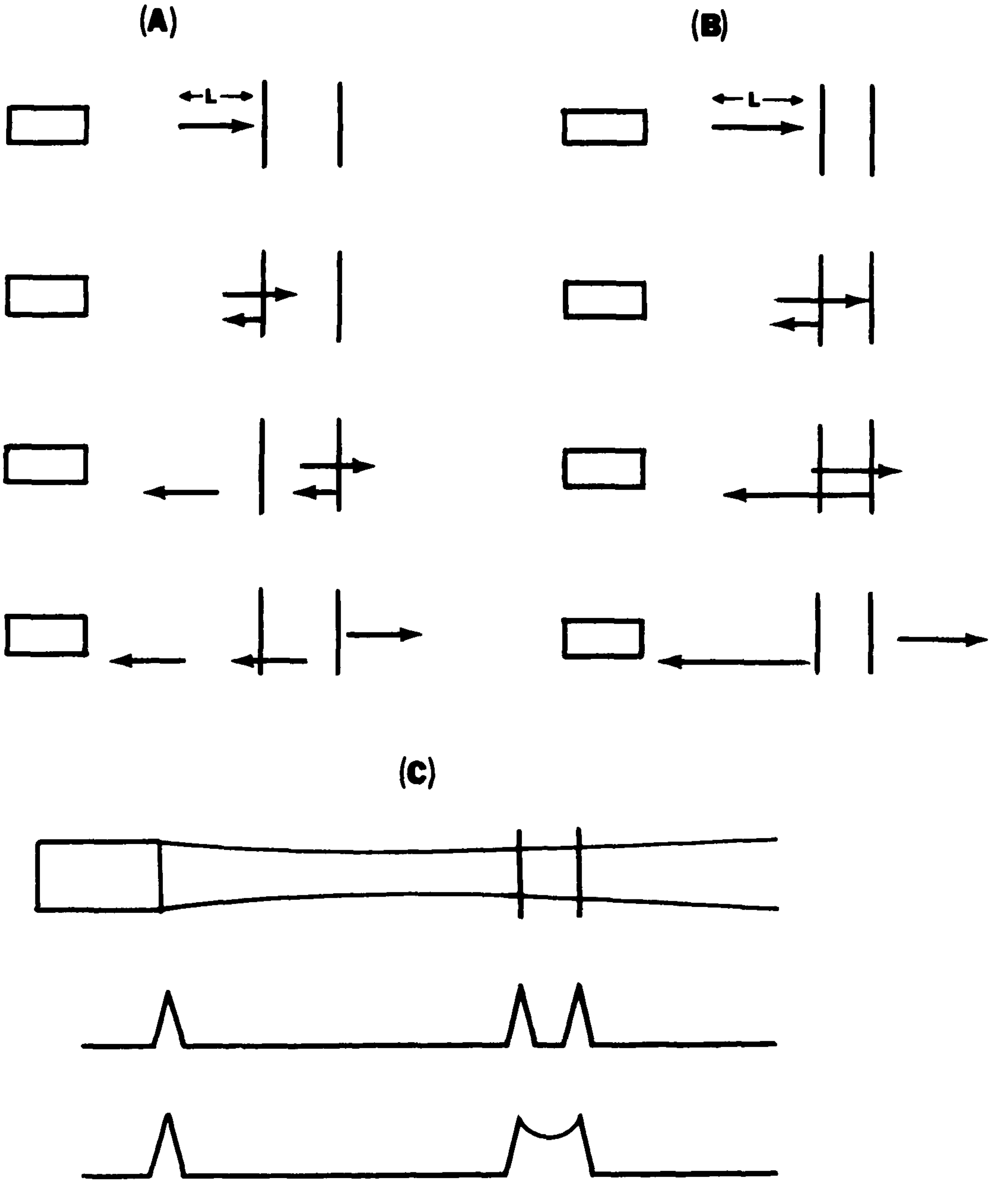


Figure 4. Effect of pulse length on axial resolution. Echoes from (A) are separated by at least one-half the pulse length L and thus are interpreted as two echoes. Echoes from (B) are closer than one-half L and are interpreted as a single echo (Bartrum and Crow, 1977). This would be viewed on an oscilloscope screen as shown in (C)

pulse length short enough for good resolution and to maintain as narrow a frequency spectrum as possible. This permits discrimination of the frequency of interest.

To predict the limits for resolution of any system, one must know the length of the pulse as well as the operating frequency. Typically, a pulse would last approximately 1 μsec. When this is multiplied by the average velocity of the ultrasonic signal in soft tissue (1540 m/sec), the pulse length is determined to be 1.54 millimeters. According to physical principles, the axial resolution can be no better than one-half the pulse length (Figure 4). Thus, a 1 μsec pulse has a minimum resolution of 0.77 mm. In addition to this, physical principles also state that axial resolution cannot be any better than one wavelength (Table 1).

Table 1. Wavelengths of commonly used ultrasonic frequencies

Frequency (mHz)	Wavelength (mm)
1.00	1.50
1.60	.96
2.25	.68
3.50	.44
5.00	.31
10.00	.15

It is easily seen, then, that as frequency increases so does the resolution. Again, there is a tradeoff in that echoes are attenuated approximately 1 dB/cm/mHz. So a high frequency would not be able to penetrate as deep as a lower frequency. In actual practice, neither of these factors is as important as the limited resolution of the instrument processing the echoes. For instance, the mechanics of the electron beam in a cathode ray tube may not allow resolution as high as the ultrasonic signal itself would allow (Bartrum and Crow, 1977).

A-scan

The simplest technique and that which is used in this experiment is called A-mode or amplitude mode (Figure 5). This employs a transducer to send and/or receive signals. If a typical pulse length is on the order of 1 μsec and the pulse repetition frequency (PRF) is 1000 pulses per second, then there is 1000 μsec between each pulse during which time the transducer can act as a receiver. Since the piezoelectric crystal in the transducer transfers a pressure wave into tissue, it also can receive pressure waves back from a tissue and produce an electrical difference across the crystal which is then monitored by the instrument.

The magnitude of the echo is given by the theory of wave reflection as

$$\text{amplitude ratio} = \frac{P_2}{P_1} = \frac{\rho_1 v_1 - \rho_2 v_2}{\rho_1 v_1 + \rho_2 v_2} = \frac{z_1 - z_2}{z_1 + z_2}$$

where v = velocity of sound, cm/sec,

ρ = density of the medium, g/cm^3, and,

$\rho v = z$ = characteristic acoustic impedance, g/cm$^2\cdot$sec.

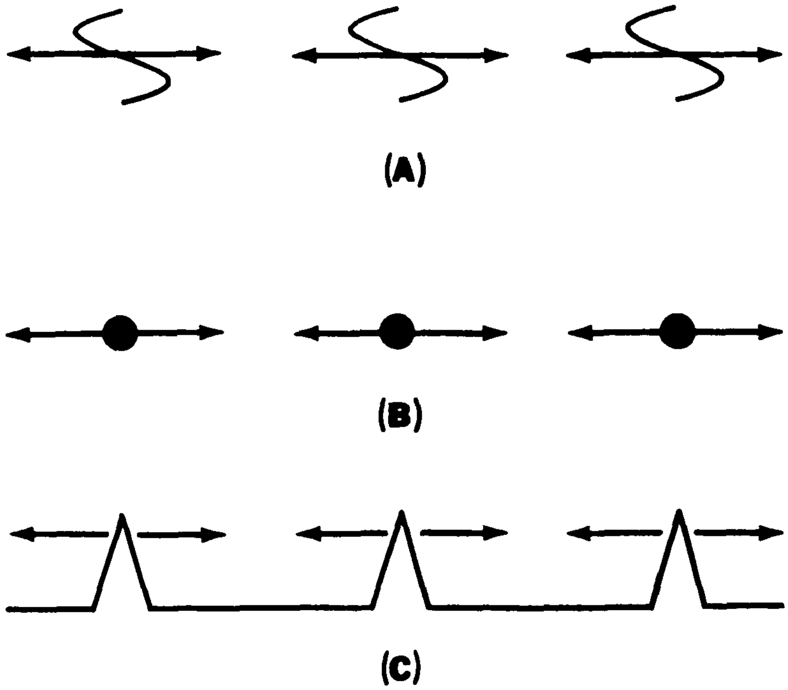


Figure 5. Principles of ultrasonic display technology are shown as time-motion, T-M (A), brightness, B (B), and amplitude, A (C) modes (Rose and Goldberg, 1979)

It can be calculated that for fat-muscle interfaces, the magnitude of the reflected echo is 10% that of the incident beam.

The transducer is pointed in the direction of interest and echoes are received from the interfaces lying in the beam path. This is a one dimensional analysis as only the echoes from the interfaces lying along the length of the beam are recorded. When an echo is received, a vertical deflection in proportion to the strength and duration of the echo occurs on an oscilloscope screen. If the speed of ultrasound in the tissue is known (Table 2), then the timebase of the instrument can be converted to the distance from the transducer to the reflecting surface. A common use of this type of scan is to examine the midline of the brain which may shift due to injury (McDicken, 1976).

Table 2. Ultrasonic velocity of soft tissues commonly tested (Johnston et al., 1979)

Tissue	Velocity (m/sec)
Water (20°C)	1483
Plasma	1571
Blood	1571
Milk	1485
Fat	1410-1479
Spleen	1520-1591
Liver	1550-1607
Kidney	1558-1568
Brain	1510-1565
Striated muscle	1568-1603
Against grain	1592-1603
With grain	1576-1587
Heart	1572

B-scan

The B-mode or brightness mode is used to produce a two dimensional view of a structure or structures. With this type of scan, the transducer is moved along or tilted within a particular plane of interest. An echo produced within that plane shows up as a bright spot rather than a vertical deflection on the oscilloscope screen (Figure 5). In this case, the position of the transducer as well as the echo must be tracked by the oscilloscope screen. When the transducer remains at a fixed angle but moves along a plane, a linear scan is produced. When the transducer remains at one point but examines a plane at several angles, it is called sector scanning. With arc scanning, the transducer moves along a single plane while remaining perpendicular to the surface it follows. Compound patterns combine sector scanning with either of the other two basic patterns, linear and arc scanning. Figure 6 illustrates these various scanning patterns (Goldberg et al., 1975). If any of these procedures are done rapidly enough to give a complete image continuously during an examination, the result is a real-time B-scan.

Gray scale B-scans incorporate logarithmic amplification of echoes. In logarithmic amplification, weak echoes are amplified more than strong echoes. The result is much better visualization of the structure being examined.

T-M scan

The last type of scan to be discussed is the T-M mode or time-motion mode. With this type of scanning, those dots normally illuminated in a B-mode representation for a fixed transducer are allowed to vary as a function of time (Figure 5). An image of a moving target for A- or B-mode will produce a fuzzy or blurred display while the T-M scan produces an indication of the echo position with respect to time (Rose and Goldberg, 1979).

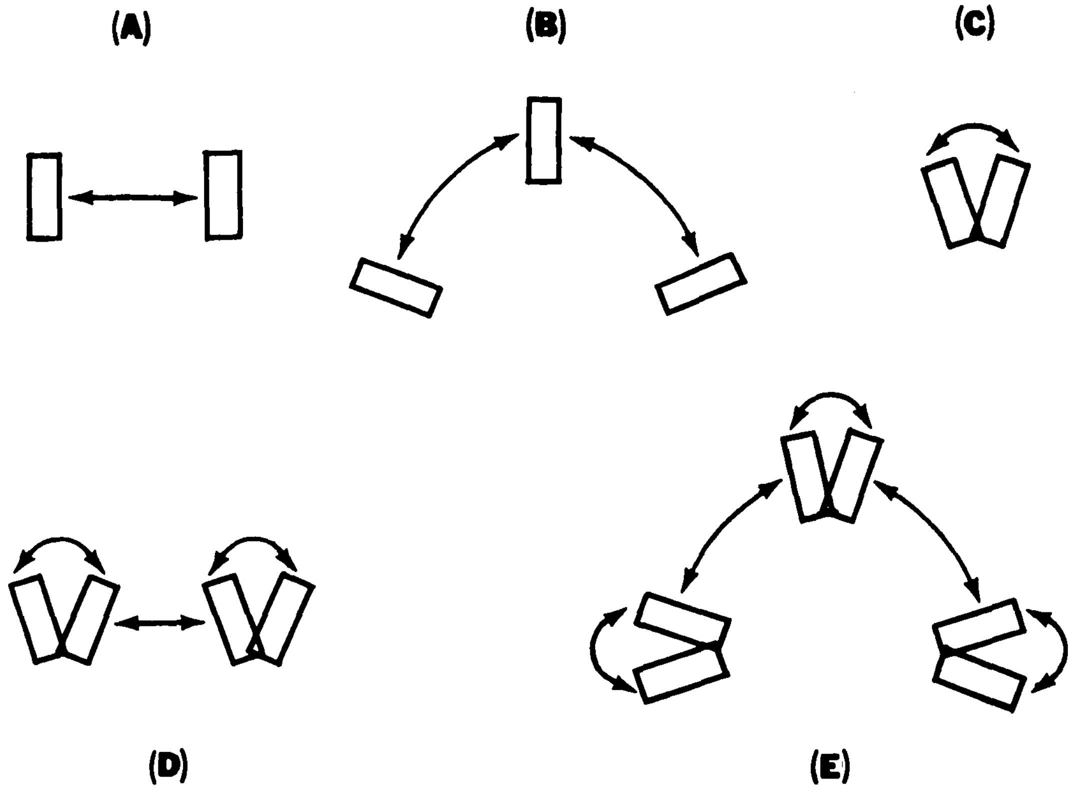


Figure 6. Transducer scanning patterns illustrated are linear (A), arc (B), sector (C), linear-sector (D), and arc-sector (E) (Goldberg et al., 1975)

Statistical Analysis

A multivariate analysis of variance, which optimized the accuracy of this system, was subsequently found to have been successfully used in a diagnostic system similar to the current study (Lerski et al., 1981). To understand the necessity of this statistical method, the following quote is given:

"The problem of classification arises when an investigator makes a number of measurements on an individual and wishes to classify the individual into one of several categories on the basis of these measurements. The investigator cannot identify the individual with a category directly but must use these measurements. In many cases, it can be assumed that there are a finite number of categories or populations from which the individual may have come and each population is characterized by a probability distribution of the measurements. Thus, an individual is considered as a random observation from this population. The question is: Given an individual with certain measurements, from which population did he arise?" (Anderson, 1958).

Multivariate analysis is unlike most commonly-used statistical analyses. For instance, in multiple regression there are simultaneous observations on several variables. Yet only one of these, the dependent variable, is regarded as a random variable. Multiple regression is, therefore, a univariate technique. This uses the predictor variables to classify the data in a way similar to qualitative classifications in an experimental design.

Multivariate analysis uses several techniques appropriate for those situations in which the random variation in several variables has to be studied simultaneously. The most widely used multivariate method is the linear discriminant function. This technique allows data from an unknown group to be assigned to the most appropriate group. It can be described by four groups of individuals, with three rotational aspects in each group. For each individual, the variables (Fourier transforms) are determined. This function will then allocate any new data to one of the four groups based on the ultrasound characteristics.

There are four assumptions made in this statistical analysis: 1) there are four independent populations, 2) it is possible to linearly separate the populations, 3) the observations used to determine the discriminant function are from a normal population, and 4) all 20 components (Fourier values) used to generate a single observation will not be independent.

The discriminant function itself is given by:

$$D_i^2 = (x-\bar{x}_i)'S^{-1}(x-\bar{x}_i)$$

where x = unknown observation,

$\bar{x}_i$ = mean of all observations in that group,

S^{-1} = covariance matrix, and

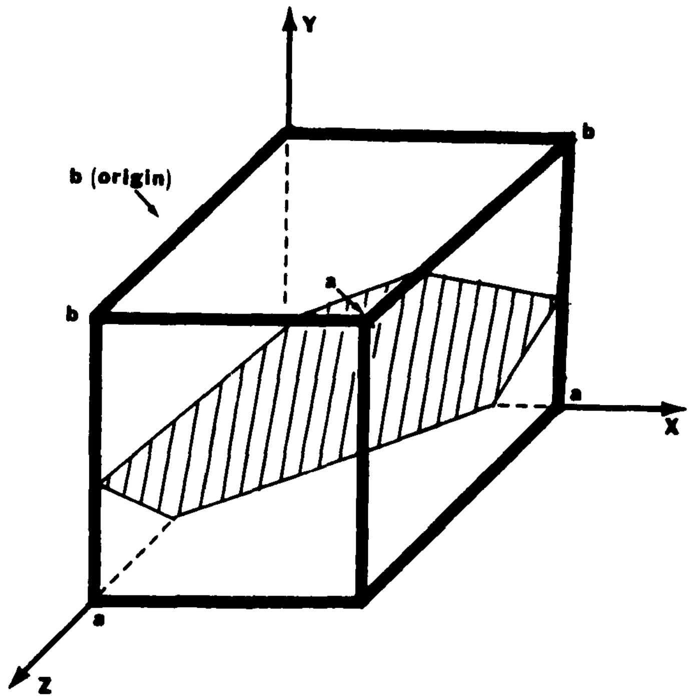
D_i^2 = separation of unknown x from $\bar{x}_i$.

Then x is assigned to population i if $D_i^2 = \min [D_1^2, \ldots\ldots D_k^2]$. If S^{-1} were not present, the result would be a simple Euclidean minimum distance classifier.

The discriminant function used in this experiment actually divides up space into four linearly separate regions. In order to predict these regions, Fourier components are utilized to form a vector. That vector is a single observation. In effect, the function is actually dividing multi-dimensional space into four regions. Each point in multi-dimensional space is compared to the rest of the points in that group. A region of space is then defined as being around that mean. An unknown observation is then plotted in multi-dimensional space and that point is compared to the four means. The region that the unknown observation is assigned to corresponds to that region as defined by the minimum D_i^2.

This can be visualized in a much simplified fashion in three-dimensional space rather than in multi-dimensional space. In Figure 7, the plane which bisects the cube is the discriminant function. This would be determined from a normal population consisting of two groups. Given a set of three-dimensional vectors, a point is located using the X, Y and Z axes. That point will be located in one of the two regions predetermined by the average spatial separation. If that point falls on the bisecting plane, the distances between the groups are equal and there is no way to tell to which group it really belongs. By merely viewing the numerical vector sets, it is very difficult to determine whether they belong to the same or to different populations. That is the purpose of the discriminant function.

The discriminant function is not used to say whether distinct Fourier components or groups of components are present in each of the four groups.



(A)

x	y	z	x	y	z
0	0	1	0	1	1
1	1	1	0	0	0
1	0	0	1	1	0

(B)

Figure 7. Simplified example of how the discriminant function will group the random variation of several variables. Two sets of 3-dimensional vectors (B) can be linearly separated by a plane such as is shown in (A). The two sets of vectors are shown as points (a) and (b)

The classification accuracy is simply how well the discriminant function can separate the groups. The best that can be hoped for is that the initial assumptions are correct and that the data used to calculate the discriminant function is from a normal population. This being true, then unknown data may be correctly classified. Due to the complicated mathematics involved, no one has found a way to predict a confidence level. The discriminant function only helps to minimize the possibility of misclassification.

In all applications of discriminant functions, the original classification of meat into groups must be made independently of the ultrasound information. The original classification is reliable but very tedious (chemical extraction). Then it must be determined how reliably the same diagnosis can be reached using variables (ultrasonic signatures) which are less costly and less tedious to gather (Armitage, 1971). In summary, this statistical method utilizes vector analysis to linearly separate (in space) the grouping of these vectors and, as such, correctly classify the grades of meat.

EXPERIMENTAL PROCEDURE

Physical and Electrical Configuration

Quality grading was subjectively evaluated according to U.S.D.A. standards. In order to accurately acquire a complete spectrum of marbled beef, all samples were obtained two days following slaughter of the animal. At this time, a qualified meat grader selected those carcasses which would most likely match each of the four grade levels. All carcasses were cooled to 4°C immediately following slaughter.

Muscle (longissimus dorsi) samples 5 to 7 cm thick were removed from the anterior portion of the beef quarter split between the 12th and 13th ribs. Samples were then stored in air evacuated plastic bags at 4°C until utilized. No sample was retained longer than two weeks. As suggested by Shung and Reid (1977), a storage temperature of 4-6°C does not change the backscatter coefficient for at least seven days. Using air evacuation storage techniques, there is no gross morphological changes greater than 0.75 mm for a minimum of two weeks.[1]

Three longitudinal cores, 32 mm in diameter, were removed from each muscle sample. Central, medial and lateral cores (Figure 8) were each incised with a razor blade prior to removal. In order to maintain consistent orientation in the ultrasound tank, the original position was marked by a razor cut.

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[1]Private communication courtesy of R. Petersohn, Meat Laboratory, Iowa State University, Ames, Iowa.

The tank design in this experiment is similar to that described by Lele et al. (1976). The experiments were performed in a 12 cm anechoic tank of 0.85% saline. Two adjustable transducer fixtures were positioned next to the outside of the tank. Each fixture could accurately be moved in 1° increments along an arc centered on the tissue sample. A permanently mounted protractor was used to determine angles. The tank itself was also free to rotate independent of the tranducer fixtures.

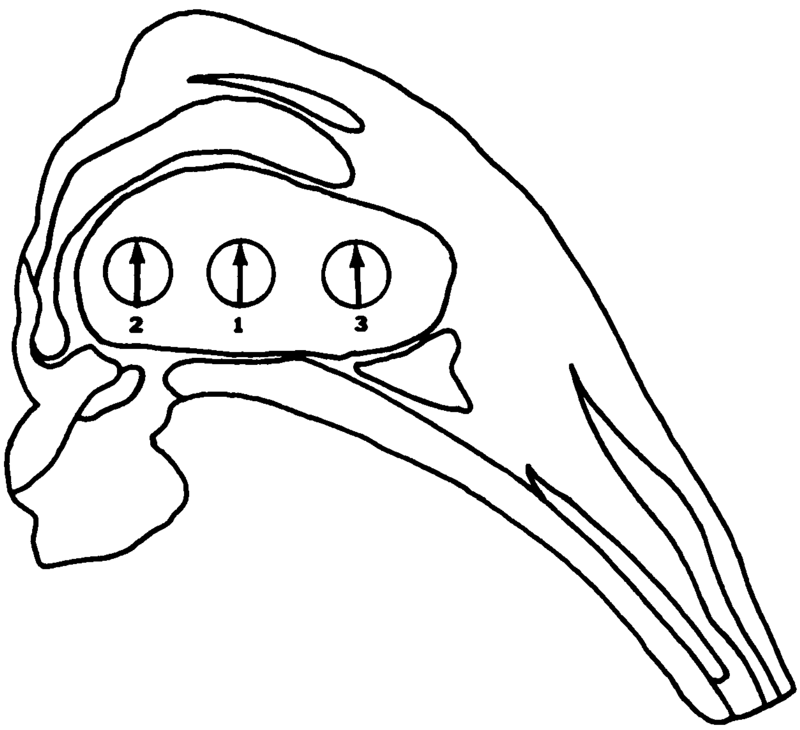
Windows were cut in the side of the tank to eliminate the tank wall as a barrier to the ultrasonic waves. Polyethylene (.002 inch thickness) lined the tank and was oil coupled to the transducers.

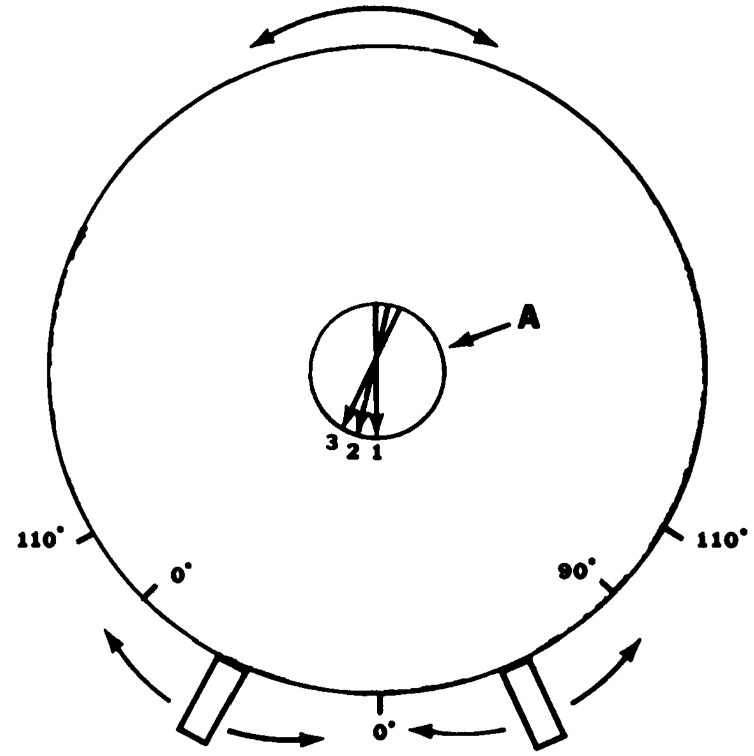
Muscle samples were centered in the tank, supported by fine (.002") steel wires placed 0.5 inches apart. This wire arrangement resulted in minimal ultrasonic wave scattering in the tank. Each specimen was ultrasonically examined in three rotational positions. Each rotation was separated by 10° (Figure 9). These rotational aspects are analogous to angles that would normally be accessible in vivo.

The experimental procedure was initiated such that tissue samples were ultasonically examined in a horizontal as well as a vertical position. The core samples were tested at 4°C, 22°C and 37°C to ensure that temperature attenuation would not adversely affect the results. These cores were also initially checked for total sample attenuation. This was done with the aid of a Plexiglas standard and the use of a pulse transmission method. In these experiments two modes of detection were utilized. For backscatter detection, echoes were received at angles other than 180°. For the transmission mode,the transmitter and receiver were axially aligned.

Figure 8. Location of central (1), medial (2), and lateral (3) incised cores within longissimus dorsi muscle. Direction of arrow indicates axis of razor cut used for orienting samples in the ultrasound tank

Figure 9. Configuration of ultrasonic tank. External degrees indicate total transducer angle of two transducer system. Internal angles correspond to single transducer system. Core sample (A) indicates the rotational positions (top view)


2
1
3


A
3 2 1
110°
0°
90°
110°
0°

Transducers (19 mm diameter, 2.25 mHz) were mounted such that for all transducer angles, the ultrasound beam remained perpendicular to the muscle fibers. This particular orientation produced the maximum backscatter amplitude signal. The transmitter PRF was set at 1000 pulses per second. The damping ratio of the instrument was found to be quite low ($\zeta = 0.05$). This resulted in a narrow-band frequency.

Data Collection

Three scans were completed at each of the three rotational positions. A total of 46 data points (integrator values of backscatter echo amplitude) were collected for each scan. For the two transducer system (double-angle scanning), data were collected at 2° increments from 20° to 110° total transducer angle. For the single transducer system (normal incidence scanning), 46 data points, each separated by 2°, were collected over a 90° angle centered on position 1 only. Total sample points were in excess of 39,000.

Ultrasonic sampling was performed with a Renco Preg-Alert.[1] The instrument was specially modified to accommodate a double or single transducer system. In addition, it included an electronic window, gated to allow integration over any portion of the signal. A schematic of the circuit is shown in Figure 10.

At every 2° increment, the ultrasonic scattering or backscattering was electronically sampled and integrated. To eliminate surface echoes,

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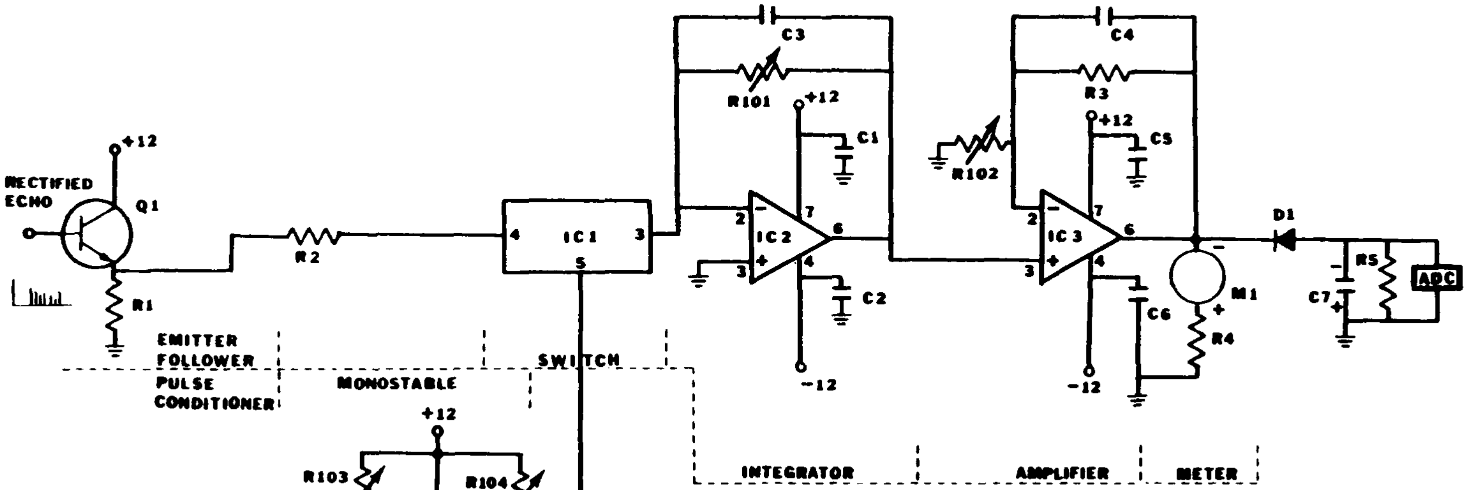
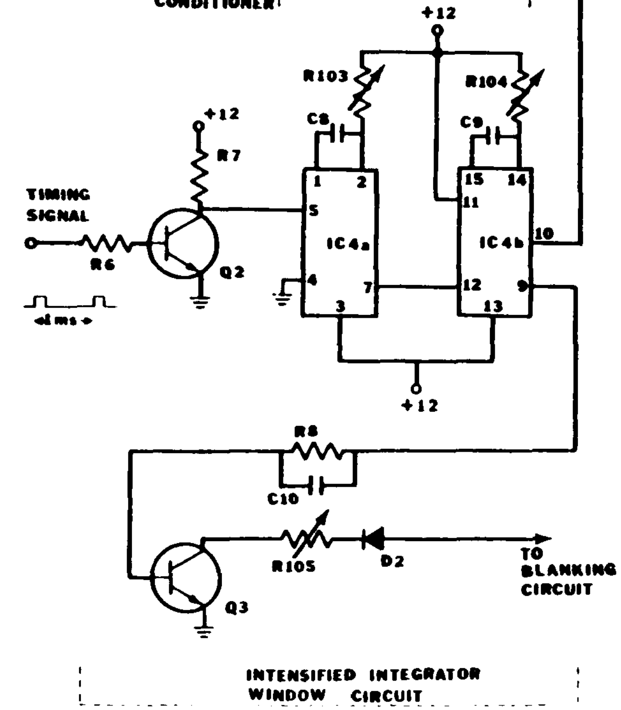
[1] Renco Corporation, Minneapolis, Minn.

Figure 10. Gating and integrator circuitry added to the Preg-Alert





IC1	4066 Bilateral Switch	R5	1M
IC2	3140 CMOS OP AMP	R6	22K
IC3	3140 CMOS OP AMP	R7	10K
IC4	4528 Dual Monostable	R8	47K
Q1-Q3	2N3414 NPN	R101	50K T.C. Adj.
D1	1N 118 Germanium	R102	10K Sensitivity
D2	1N 4148 Silicon	R103	20K Window Position
M1	Ammeter 0-1 ma	R104	250K Integrator Window Size
ADC	to computer analog to digital input	R105	50K Brightness
R1	22K	C1-C6	.01 mf
R2	4.7K	C7	1 mf
R3	22K	C8	.01 mf
R4	1K	C9	470 pf
		C10	68 pf

only the signal from the central 15 mm portion of the tissue was integrated. The integrator output was collected and stored through an A/D converter on a PDP8-E mini-computer.[1] Each of the three rotational positions was scanned three times to average out any minor off-angle inconsistencies. The average ultrasonic signature (graph of the relative integrator values versus the transducer/receiver angles (2θ)) was computed and displayed by the computer. This signature corresponded to the average backscatter pressure at each sample angle. Since it is possible to saturate the integrator with any particular tissue echo, the gain setting was adjusted for every muscle specimen. Using a standard reflector, the numerical differences in each gain setting were calculated. These values were later used for mathematical standardization.

The ultrasonic signatures were composed of waveforms of many different spatial frequencies. It was necessary to reduce the volume of data collected. The most practical method of data reduction is by Fourier transform analysis. This reduced the signatures to the amplitude of each individual spatial frequency component. This technique required equispaced data with $D(0) = D(M)$ for an exact period. The data points began with $D(1)$ and ended with $D(M) = D(0)$. For this technique, $D(0)$ is the initial reference value such that the computer would utilize a complete set of data. The last variable, $D(M)$, is equal to the reference value with M as the number of values in that data set. Since the averaged data seldom fit this criterion, a forced fit was generated by two separate

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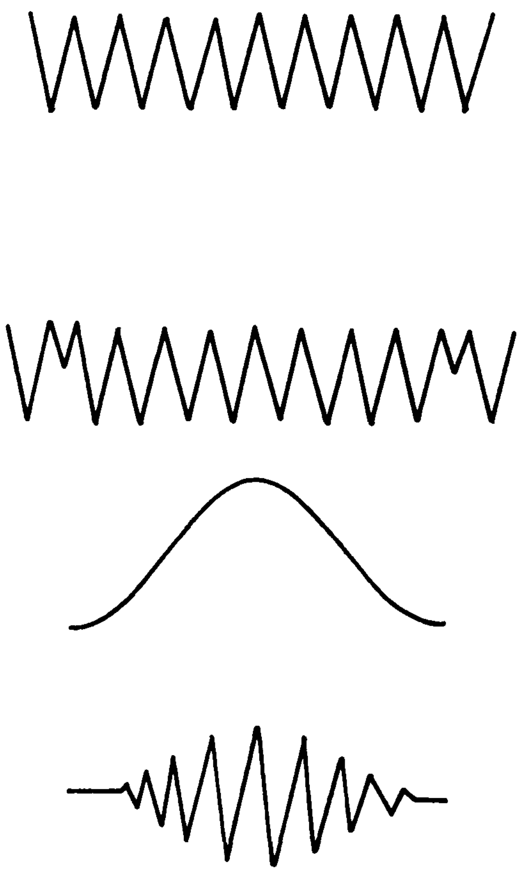
[1]Digital Equipment Corporation, Maynard, Mass.



Actual input (A)

Assumed input (B)

Window function (C)

Windowed input (D)

Figure 11. Most problems in analyzing an input (A) occur at the nonperiodic time records edges (B). Therefore, a window function (C) forces the Fourier transform to concentrate on the time record's center, producing a more valid assumed input (D) (Gibbs, 1981)

methods. The first method utilized a $\cos^2 \theta$ function such that the first and last data point was forced to zero. The effect of this type of function is shown in Figure 11. As is illustrated, the frequency of the windowed function does not change as long as the original data are of sufficiently high freqquency. The second method created a left-to-right mirror image of the data. If low frequency information was lost by the first method, or if high frequency information was artificially created by the second method, the final results would not match.

Nyquist theory dictates that at least two data points must be present to detect a single frequency. This reduces aliasing effects. With 46 original data points available, at best only 23 spatial frequency components can be produced by the Fourier transform analysis. In practice, it is not likely that the maximum and minimum values would be detected for all 23 spatial frequency components. For this project, the first 20 harmonics were retained. As will be shown later, only the first ten harmonics were statistically required.

Lipid Extraction

Following ultrasonic examination, each meat core was pulverized in the presence of liquid nitrogen. Aliquots (13-15 g) of each sample were retained for lipid extraction. Chemical analyses were conducted according to Association of Official Analytical Chemists (1980) procedures.

RESULTS AND DISCUSSION

Three Dimensional Configuration

It was proposed, prior to initiation of this project, that as Bragg scattering can appropriately be used in X-ray crystallography to calculate distances between successive planes of atoms, then marbling may be an acceptable configuration for ultrasonic utilization of this technique. The problem may be that this procedure is typically applied to an ordered, or at least semi-ordered, structure. As no information is available as to the 3-dimensional structure of marbling, a test for planar or globular arrangement was devised. If planar was the result, then what is the orientation aspect of the planes?

It seemed that depending on the particular piece of meat, planar or globular structures could be visually ascertained (Figure 12). If one particular orientation yielded better results, then treatment could be allowed as an average planar configuration. If no particular orientation differences were detected, a globular configuration would be assumed. Either way, for at least a semi-ordered structure, the average spacing would be determined.

Tissue sample cores were aligned horizontally and vertically in the examination tank. Ultrasonic scans were completed on each of the three rotational positions. An example of the initial ultrasonic signatures is shown in Figure 13 through Figure 18. From a visual assessment

Figure 12. Illustrations of the lower limits of certain degrees of typical marbling referred to in the official United States Standards for grades of carcass beef (obtained from Food Safety and Quality Service, Meat Quality Division, United States Department of Agriculture)

1—Very abundant	4—Slightly abundant	7—Small
2—Abundant	5—Moderate	8—Slight
3—Moderately abundant	6—Modest	9—Traces

(Practically devoid not shown)

UNITED STATES DEPARTMENT OF AGRICULTURE
FOOD SAFETY AND QUALITY SERVICE
MEAT QUALITY DIVISION

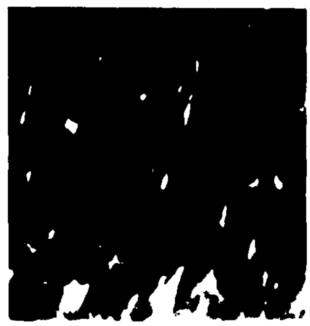
1

2

3

4

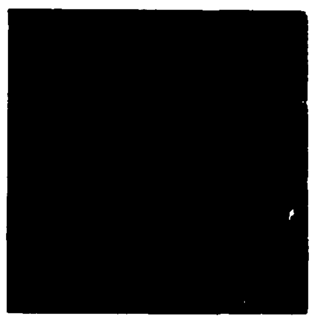
5



6

7

8



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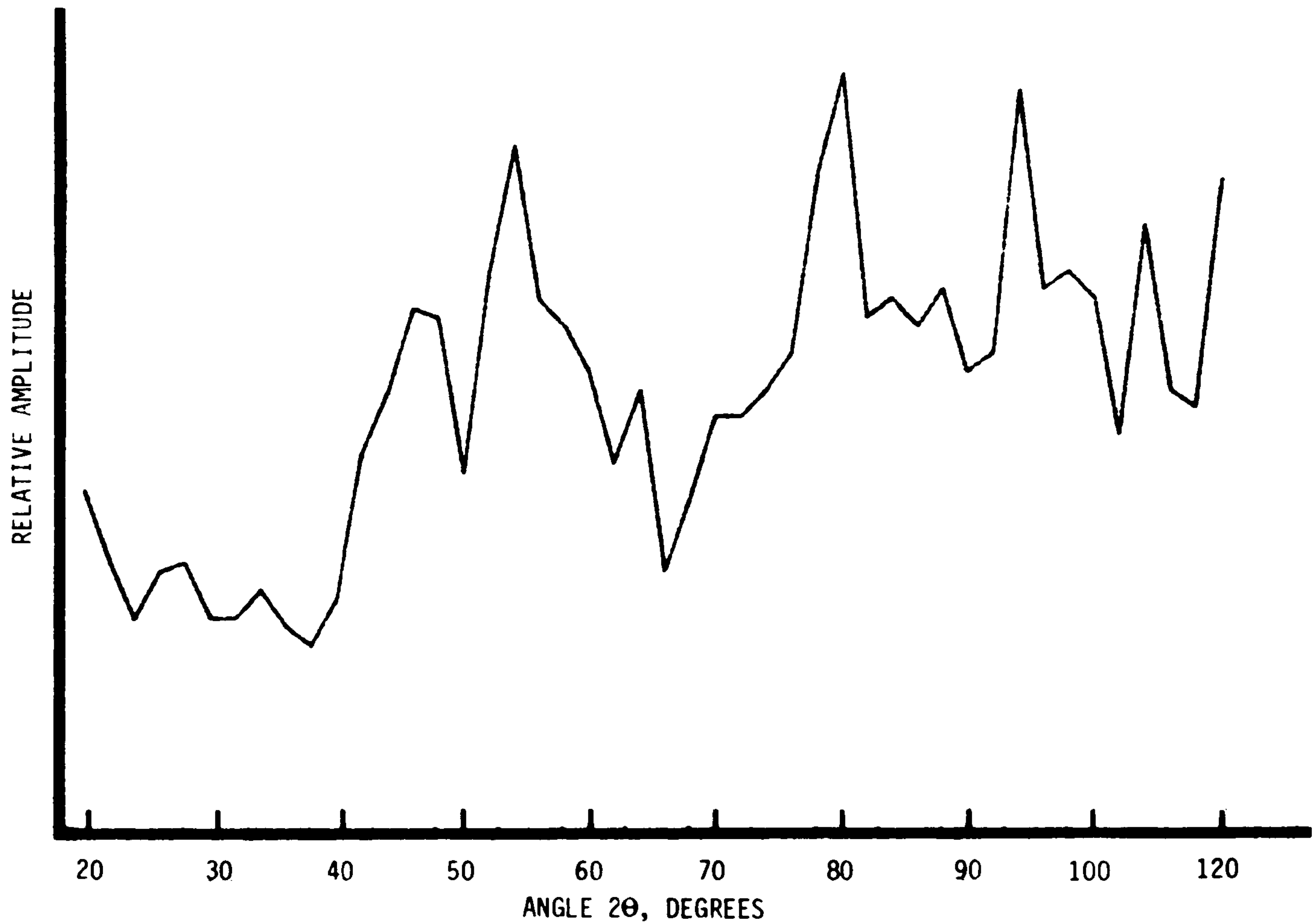


Figure 13. Illustration of ultrasonic signature from tissue sample in vertical alignment, position 1

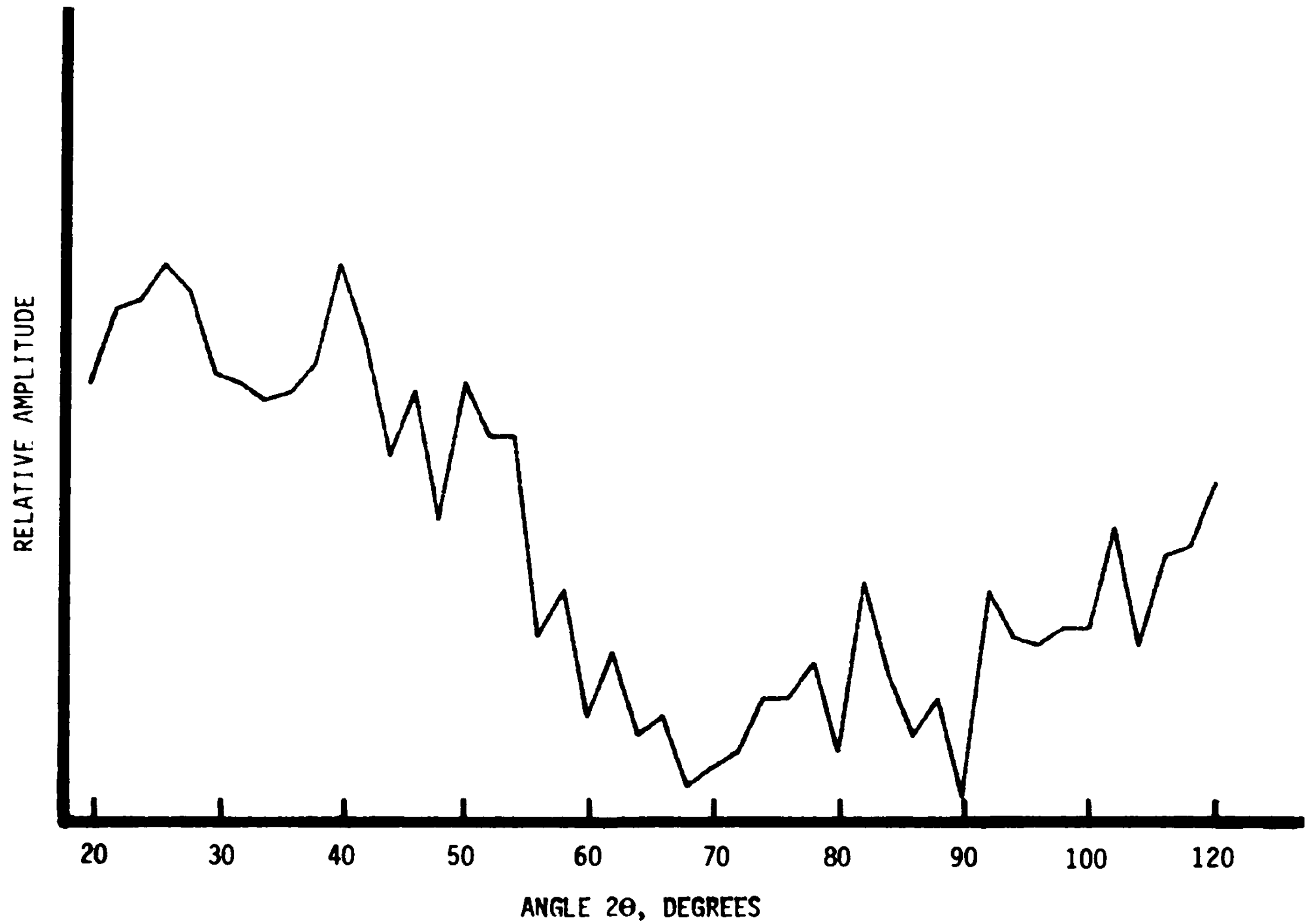


Figure 14. Illustration of ultrasonic signature from tissue sample in vertical alignment, position 2

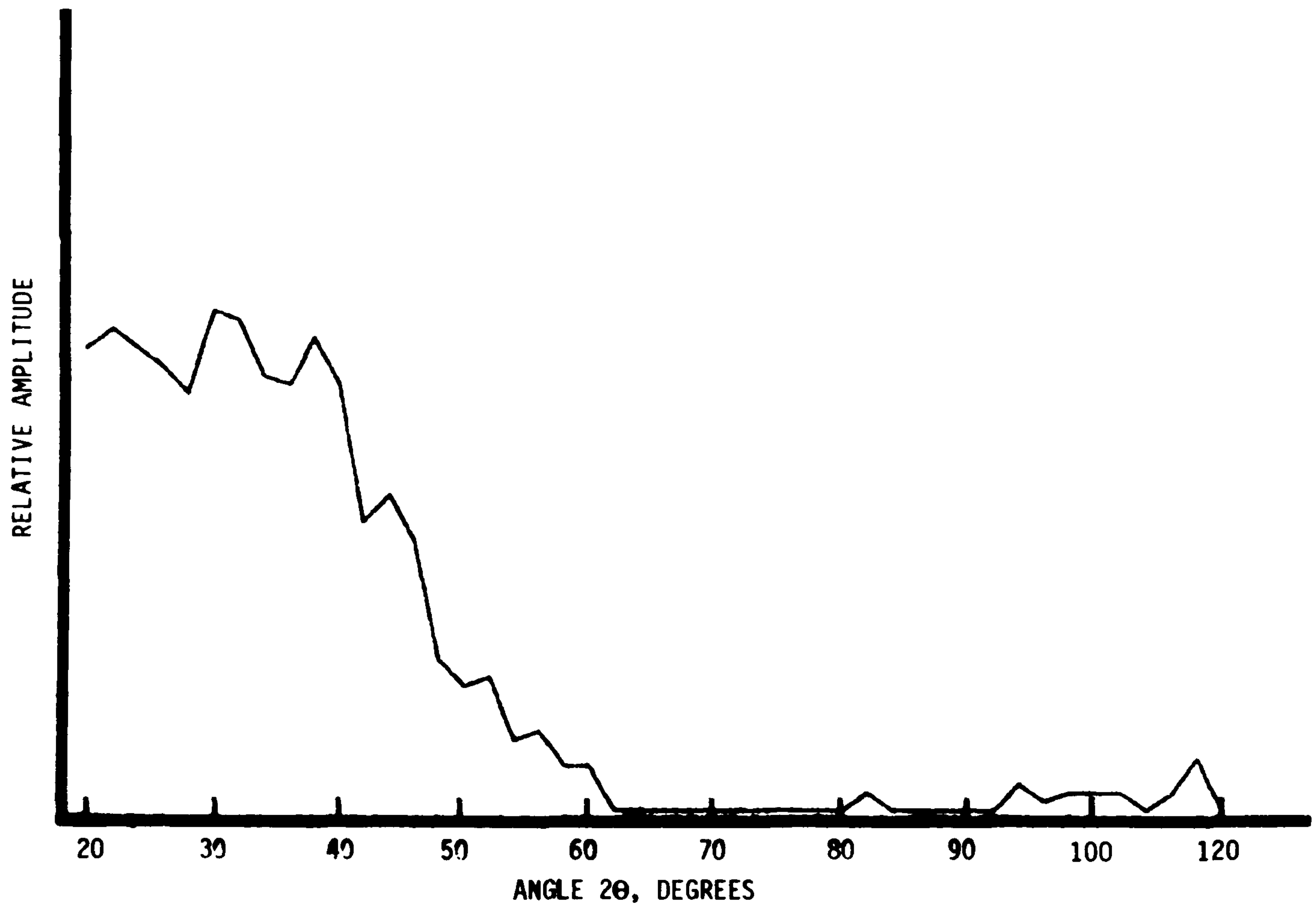


Figure 15. Illustration of ultrasonic signature from tissue sample in vertical alignment, position 3

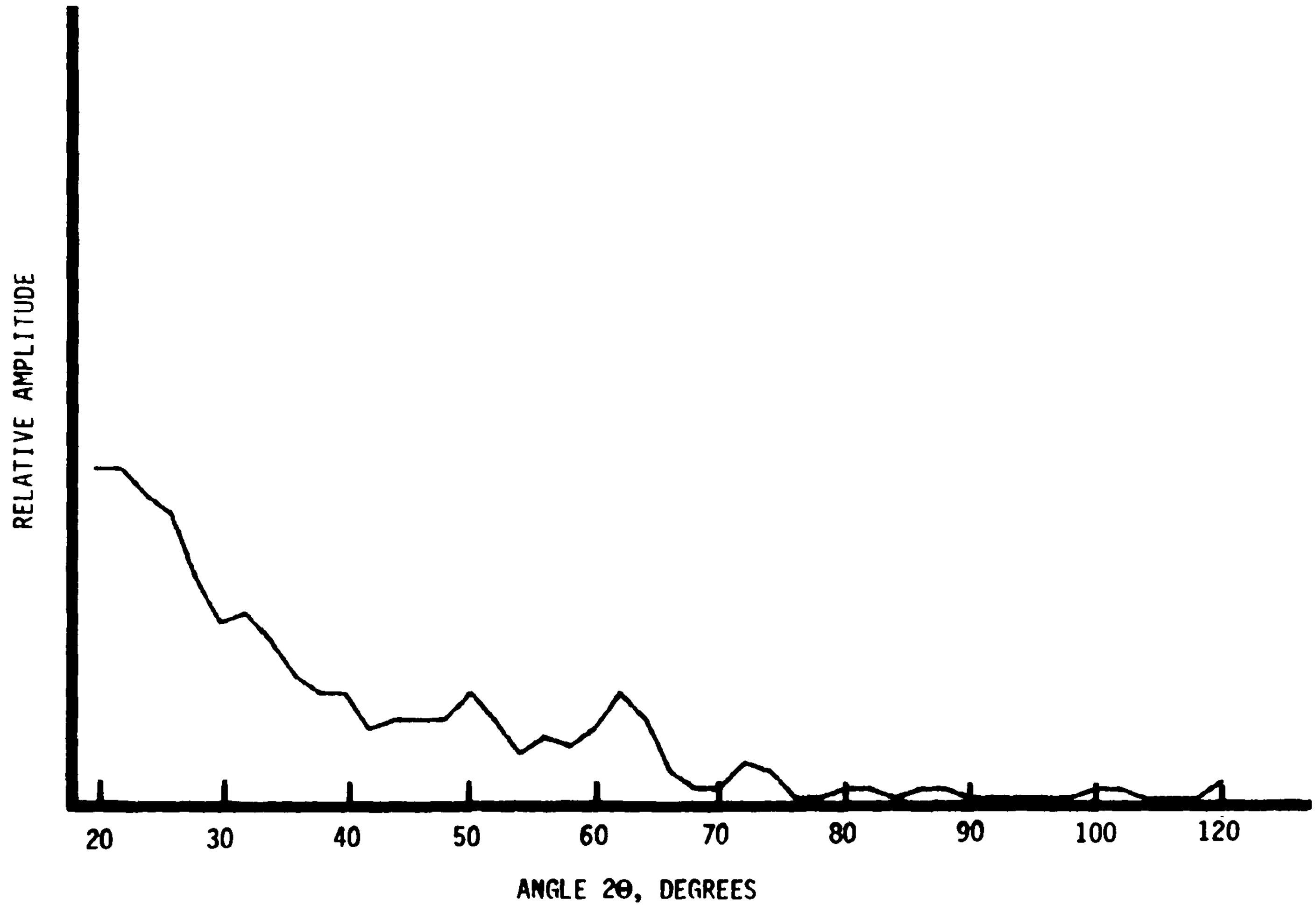


Figure 16. Illustration of ultrasonic signature from tissue sample in horizontal alignment, position 1

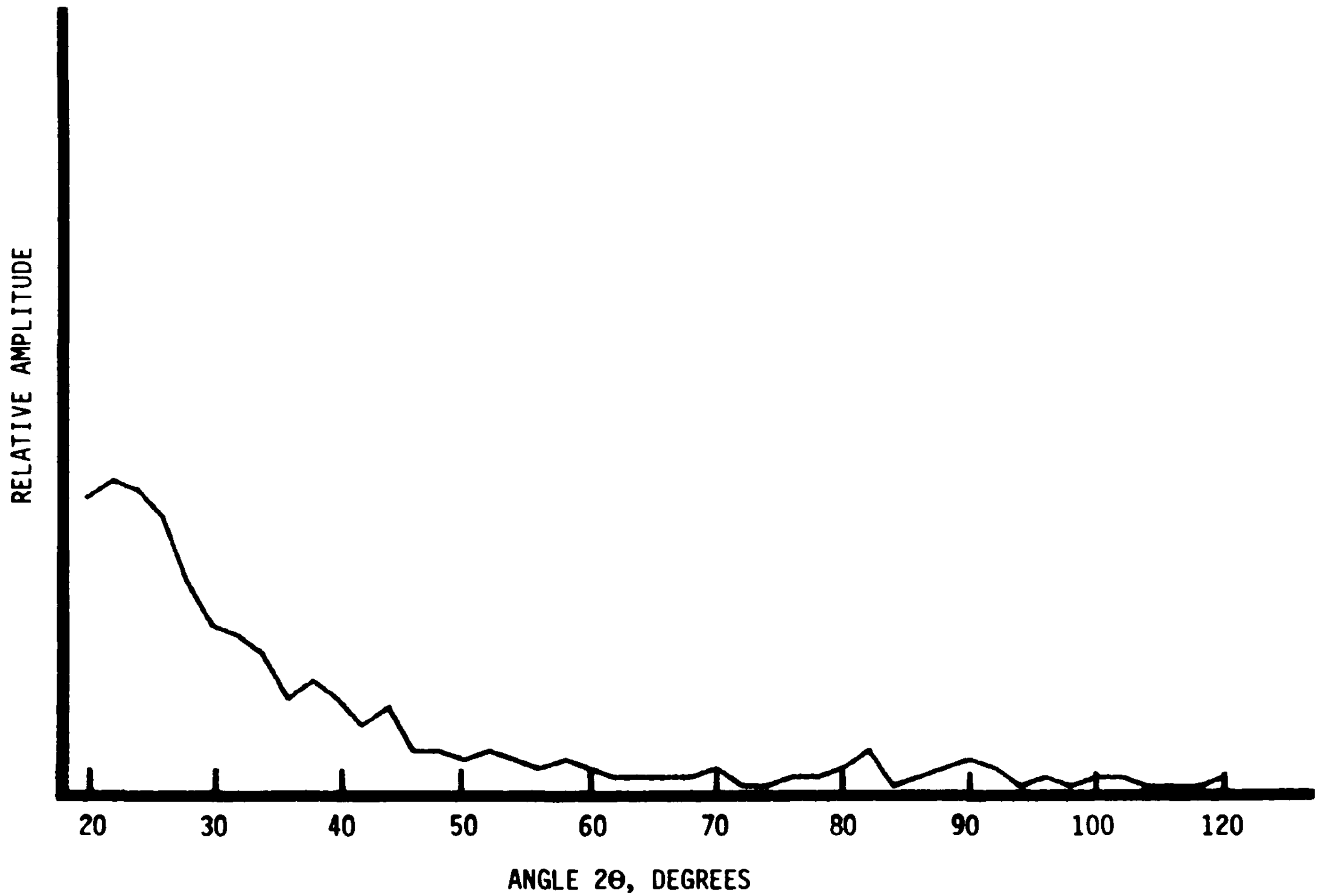


Figure 17. Illustration of ultrasonic signature from tissue sample in horizontal alignment, position 2

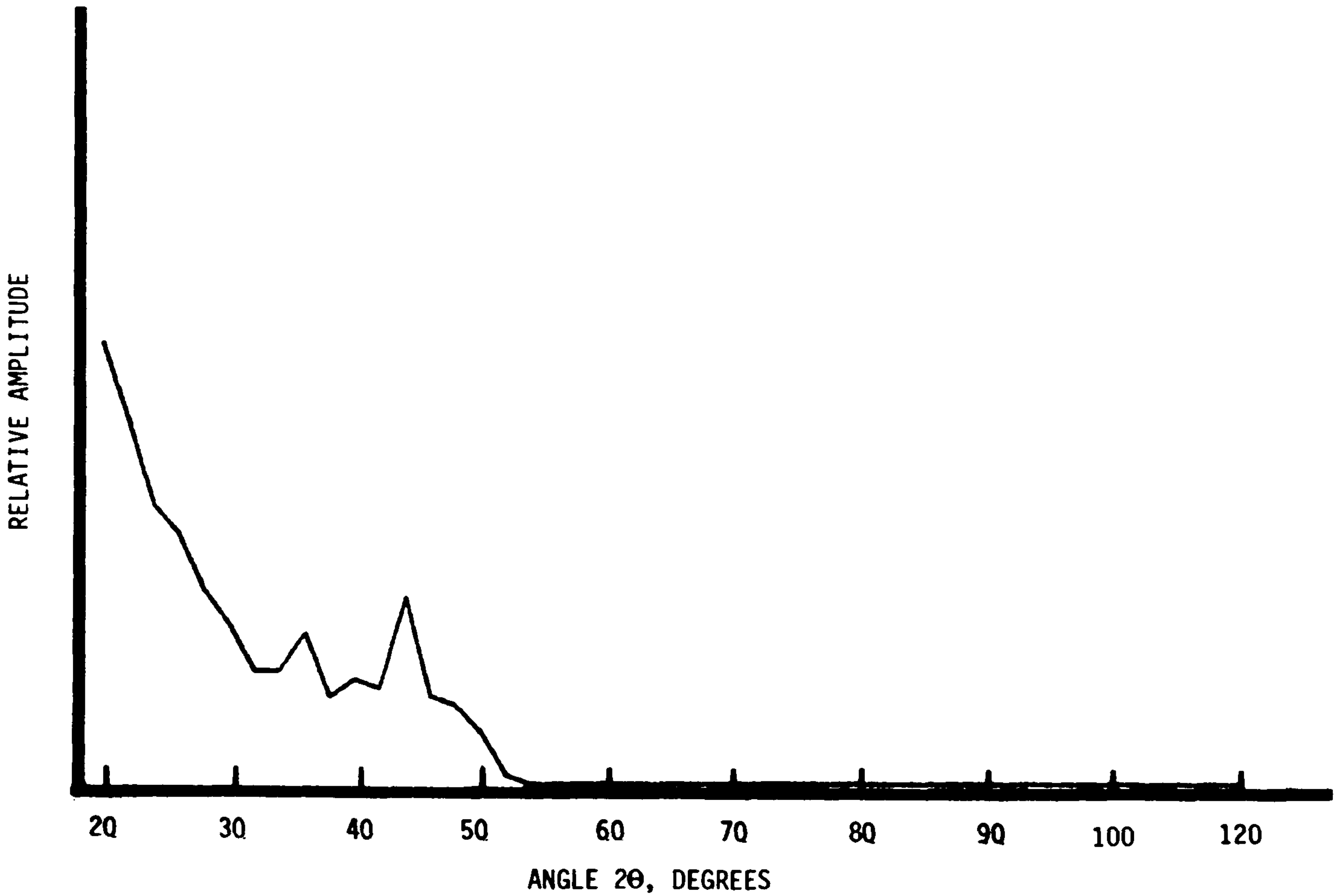


Figure 18. Illustration of ultrasonic signature from tissue sample in horizontal alignment, position 3

of the signatures, a judgment was made to proceed with the vertical alignment method. This appeared to contain the maximum spatial frequency components and conveniently permitted the highest alignment accuracy.

Temperature Dependence

Attempts to record ultrasonic signatures from refrigerated meat were unsuccessful. Anatomical and physical reasons for this were not considered. This was of concern, however, for subsequent investigation. It was not desirable to have temperature attenuation factors affecting the results.

Comparison of the ultrasonic signatures at 22°C and 37°C was undertaken to determine if the signal was significantly attenuated to warrant 37°C testing only. Initially, six core samples (three prime and three standard grade) were ultrasonically examined at 22°C and, after an equilibration period, at 37°C. Table 3 shows the result of this investigation. For each analysis, gain setting was adjusted to the same predetermined integrated echo amplitude. Although minor variations did occur, attenuation was not of significant magnitude to make room temperature analysis inappropriate. A difference of ± 1 gain factor was not judged to be meaningful. Following this investigation, all ultrasonic readings were conducted at room temperature.

Table 3. Temperature versus gain setting necessary to increase backscatter amplitude to approximately identical values

Grade	Core	Rotation	Gain setting 37°C	Gain setting 22°C
Standard	1	1	1	2
		2	1	2
		3	1	2
	2	1	4	2
		2	2	2
		3	2	2
	3	1	2	1
		2	2	1
		3	1	2
Prime	1	1	3	7
		2	2	5
		3	2	4
	2	1	7	8
		2	6	7
		3	6	6
	3	1	4	6
		2	4	7
		3	6	8

System Verification

A model of known geometry was designed and constructed to verify the electronic system. Steel pins (0.02 inches diameter) were arranged in a grid pattern of 0.1 inch spacing. Calculations were made from the formula for the Bragg scattering condition

$$n\lambda = 2d \sin \theta$$

where n = integer,

λ = ultrasonic wavelength, mm,

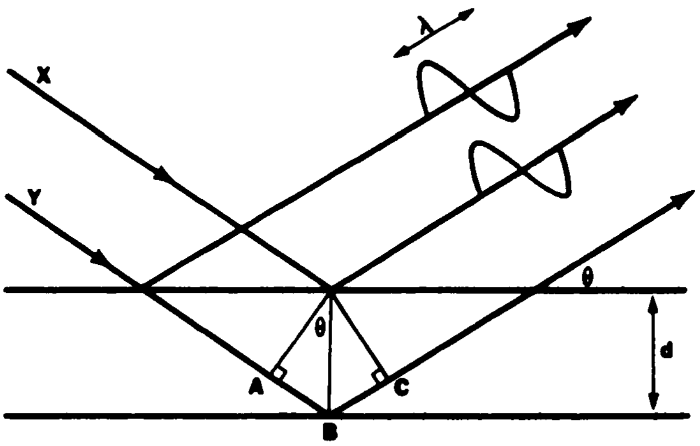
d = distance between adjacent scattering points, mm,

θ = angle from horizontal to scattered signal, degrees.

As seen in Figure 19, the path length difference (ABC) is $2d \sin \theta$. Since this must equal whole wavelengths for a strong beam condition, the Bragg law is defined.

For a 2.25 mHz signal and a grid spacing of 0.1 inches between pins, constructive interference occurs at approximately 15, 30, 46, 63, 82 and 104 degrees total transducer angle (2θ). The mathematical calculations are in agreement with that actually observed using the ultrasound system (Figure 20).

To test if the Fourier transform analysis was performing correctly, a numerical square wave of amplitude 15 was substituted into the equation. For this test, the theoretical Fourier transform values are shown with the simulated values in Table 4. For a square wave, only odd harmonics are present.



Extra path of Y = ABC = $d \sin \theta + d \sin \theta$

For constructive interference: $2d \sin \theta = n\lambda$

Figure 19. Geometry for Bragg diffraction (Lele et al., 1976)

Gain standardization was mathematically calculated with the aid of a single stainless steel pin reflector. At each gain setting, the ultrasound scan was completed three times. The average DC (Fourier analysis) value was recorded for the eight settings. Each of seven settings was then multiplied by a corresponding factor to standardize that value to the maximum gain.

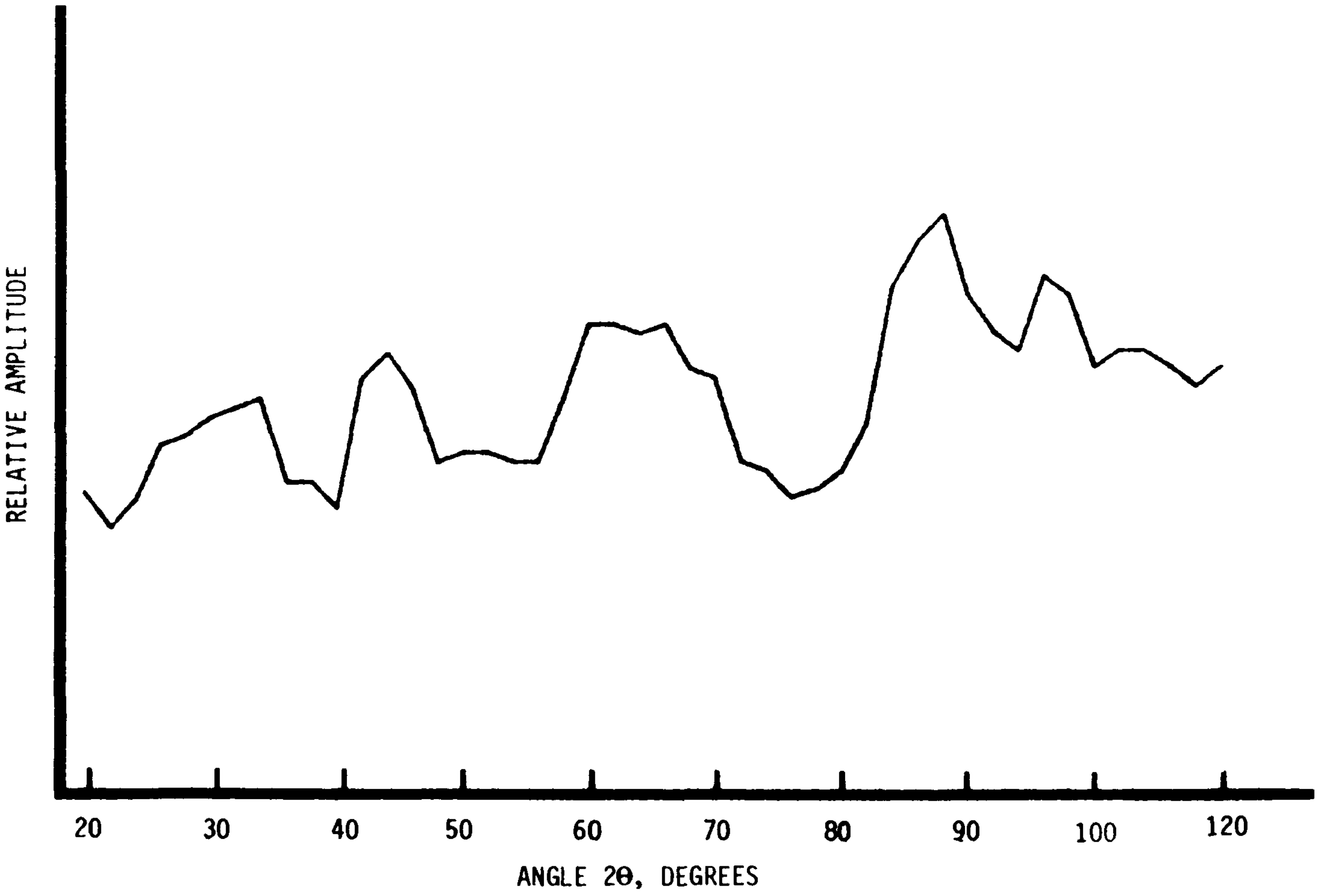


Figure 20. Ultrasonic signature from steel pin model showing constructive interference at approximately 34, 44, 62, 88, and 96 degrees total transducer angle (2θ)

Table 4. Fourier transform analysis for a model square wave of amplitude 15

(N)	C(N) Theoretical	C(N) Simulated
1	19.10	19.10
3	6.37	6.37
5	3.82	3.83
7	2.73	2.75
9	2.12	2.15
11	1.74	1.77
13	1.47	1.51
15	1.27	1.33
17	1.12	1.18
19	1.01	1.07

Normal Incidence Scanning

A single transducer backscatter analysis was performed with the core sample in position 1 only. No rotational exams were necessary as this would simply result in a lateral shift of the signature. Duplicate scans proved to be similar as would be expected with a single transducer. Only 90° scans were attempted in this format as angles larger than this would be difficult in vivo. A typical example of a signature from a single transducer system is shown in Figure 21. Classification accuracy, as predicted by discriminant analysis, is given in Table 5. The two methods used to fit the data produced somewhat different results

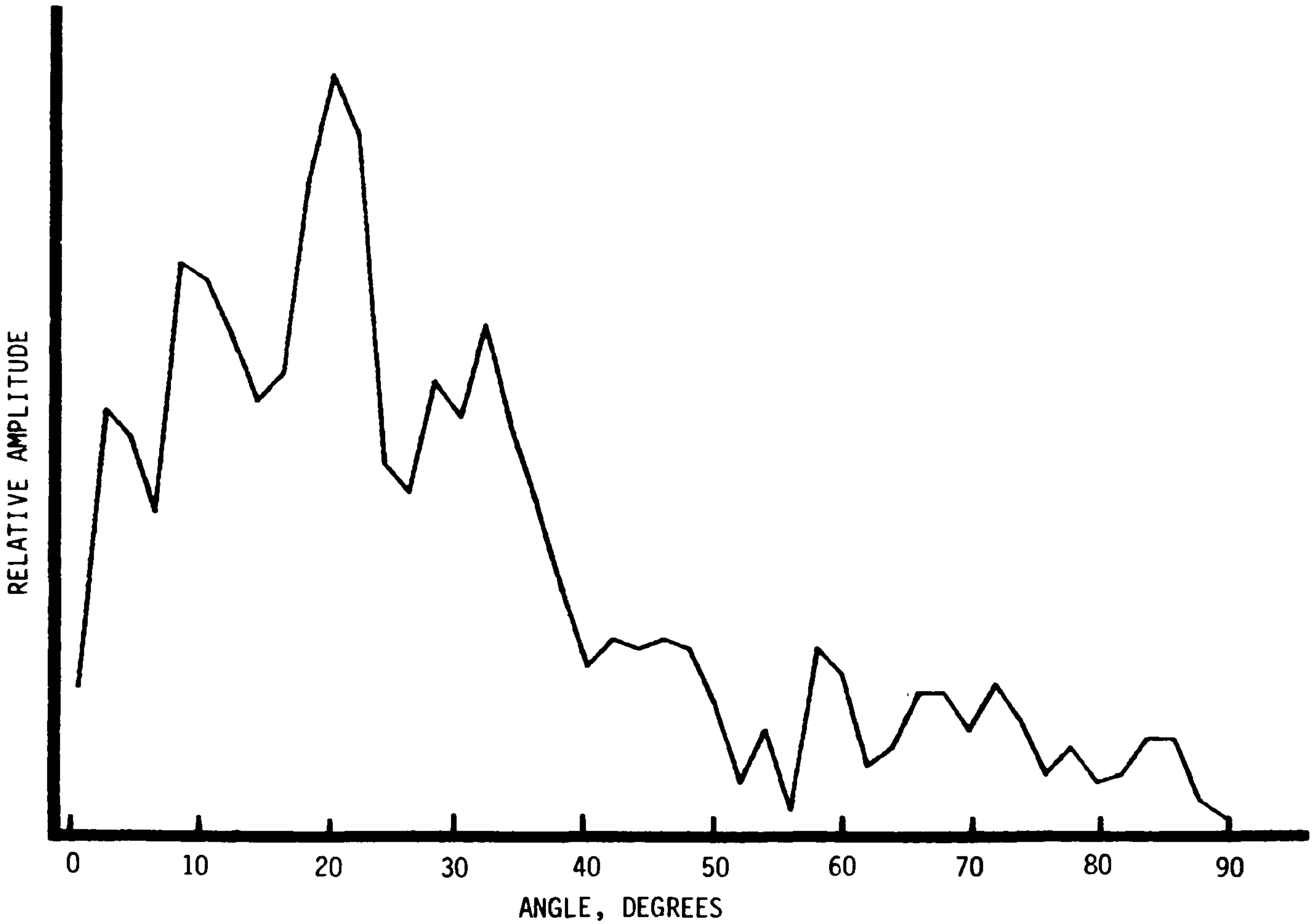


Figure 21. Ultrasonic signature of single transducer system illustrating reduced amplitude backscatter as transducer orientation varies

Table 5. Classification accuracies of single transducer system using discriminant analysis. Numbers not in parentheses represent mirror imaged data. Numbers in parentheses represent data generated by a $\cos^2 \theta$ window function. The classification accuracies are given for the first 5 harmonics, (a), and the first 10 harmonics, (b). The 10 harmonic analysis is given only to illustrate the point that classification accuracy increases as the number of variables approach the degrees of freedom. The 5 harmonic system is more reliable

From grade	Percents classified into grade 1	2	3	4	Total observations	
1	50(50)	17(9)	8(8)	25(33)	12	
2	0(8)	67(43)	8(33)	25(16)	12	(a)
3	0(8)	8(50)	92(26)	0(16)	12	
4	0(9)	8(16)	17(0)	75(75)	12	

From grade	Percents classified into grade 1	2	3	4	Total observations	
1	92(100)	0(0)	8(0)	0(0)	12	
2	0(0)	92(100)	0(0)	8(0)	12	(b)
3	0(0)	8(0)	92(100)	0(0)	12	
4	0(0)	0(0)	0(0)	100(100)	12	

under the conditions of normal incidence scanning.

By using a linear discriminant function, classification accuracy will always increase as the number of variables in the array approaches the number of degrees of freedom. This is analogous to fitting two points to a straight line. This implies a tradeoff of the statistics (i.e., reliability versus total numbers). A second tradeoff is having too few variables examined such that the results are meaningless. The optimal way to test any system such as this, then, is to have the number of observations much greater than the number of variables examined.

In the case of a single transducer system, twelve observations are a rather limited number. In compensation, higher reliability is achieved with the use of 5 harmonic analysis rather than 10 harmonic analysis. Even though 20 harmonics were calculated, they could not all be used with only twelve observations available. Table 5 illustrates the point that as the number of variables (5 or 10) approach the number of degrees of freedom (11), accuracy increases.

Double Angle Scanning

Double angle scans were conducted as previously described. The range of 20° and 110° (total transducer angle) is representative of those angles which may be achieved in vivo. Due to the physical size of both the examination tank and the transducers, angles closer than 20° were not possible.

Duplicate scans did not appear similar. This agrees with the work of Huggins and Phelps (1976) in which liver scans taken between respiration cycles were shown to be different. This was due to the movement of tissue during respiration and the fact that identical ultrasonic alignment with that same tissue is impossible from one respiration cycle to the next. The magnitude of the change for off-normal angles of incidence is directly proportional to the ultrasonic beamwidth and the degree of off-normal angulation. This is due to phase cancellations resulting from different path lengths between the transducer and target. Repositioning the core samples adversely affects any attempt at duplication.

It was proposed, then, that by reducing the ultrasonic signatures to the individual spatial frequency components, the bounds of any given grade could be established by statistical methods. Even if the ultrasonic signatures or spatial frequencies did not appear to match, they may possibly be spatially grouped. The classification accuracy as determined by statistical methods is given in Table 6 through Table 8.

Using the example of Table 6, with total observations exceeding 55, it is feasible to use all 20 harmonic variables to calculate the discriminant analysis. As with the single transducer system, highest classification accuracy is associated with the good grade. This means that of the 4 grades of beef, the good grade was the most often correctly identified. Interpretation, though, of this data can be misleading. As rotational positions were not accounted for, the primary assumption is that marbling itself has no specific orientation. As will be shown later, this is not the case.

For Table 7 and Table 8,.the rotational aspects are separated. For each of the 3 segments of Table 8, the accuracies of correct classification are very good. All accuracies on a diagonal from upper left to lower right are very high. The analysis, corresponding to 10 harmonics in particular, illustrates that marbling is spatially oriented. These same data show much improved accuracy and reliability as compared to previous data that contained no rotational separation.

Table 6. Classification accuracies of double transducer system using discriminant analysis. No rotational separation: (a) first 5 harmonics, (b) first 10 harmonics, (c) first 20 harmonics. Numbers not in parentheses represent mirror imaged data. Numbers in parentheses represent data generated by a $\cos^2 \theta$ window function

From grade	Percents classified into grade 1	2	3	4	Total observations	
1	64(59)	6(5)	25(26)	5(10)	64	
2	29(40)	14(17)	43(33)	14(10)	56	
3	26(22)	9(2)	60(74)	5(2)	57	(a)
4	33(25)	9(7)	29(33)	29(35)	58	

From grade	Percents classified into grade 1	2	3	4	Total observations	
1	45(60)	9(5)	34(28)	11(7)	64	
2	14(29)	41(30)	36(32)	9(9)	56	(b)
3	14(15)	4(3)	81(78)	2(4)	57	
4	26(14)	9(10)	24(32)	41(44)	58	

From grade	Percents classified into grade 1	2	3	4	Total observations	
1	78(81)	2(4)	20(10)	0(5)	64	
2	11(19)	57(53)	23(19)	9(9)	56	(c)
3	0(10)	2(0)	95(85)	3(5)	57	
4	10(8)	3(7)	21(12)	66(73)	58	

Table 7. Classification accuracies of double transducer system using discriminant analysis on the first 5 harmonics: (a) position 1, (b) position 2, (c) position 3. Numbers not in parentheses represent mirror imaged data. Numbers in parentheses represent data generated by a $\cos^2 \theta$ window function

(a)

From grade	Percents classified into grade: 1	2	3	4	Total observations
1	50(62)	8(4)	33(22)	8(12)	24
2	30(33)	35(34)	35(19)	0(14)	20
3	14(28)	0(0)	86(72)	0(0)	21
4	27(27)	5(6)	32(22)	36(45)	22

(b)

From grade	Percents classified into grade: 1	2	3	4	Total observations
1	45(65)	10(0)	35(25)	10(10)	20
2	22(35)	28(27)	39(27)	11(11)	18
3	22(11)	0(0)	72(89)	6(0)	18
4	33(28)	11(16)	17(28)	39(28)	18

(c)

From grade	Percents classified into grade: 1	2	3	4	Total observations
1	70(60)	10(25)	5(15)	15(0)	20
2	12(22)	65(61)	24(17)	0(0)	17
3	17(22)	17(17)	61(61)	5(0)	18
4	28(5)	6(34)	28(27)	39(34)	18

Table 8. Classification accuracies of double transducer system using discriminant analysis on the first 10 harmonics: (a) position 1, (b) position 2, (c) position 3. Numbers not in parentheses represent mirror imaged data. Numbers in parentheses represent data generated by a $\cos^2 \theta$ window function

From grade	Percents classified into grade				Total observations	
	1	2	3	4		
1	75(79)	8(4)	8(4)	8(13)	24	
2	5(14)	70(67)	20(19)	5(0)	20	(a)
3	5(0)	0(0)	95(100)	0(0)	21	
4	14(9)	5(9)	18(23)	64(59)	22	

From grade	Percents classified into grade				Total observations	
	1	2	3	4		
1	65(95)	15(0)	15(5)	5(0)	20	
2	0(27)	89(56)	11(17)	0(0)	18	(b)
3	0(0)	0(0)	100(100)	0(0)	18	
4	17(6)	6(11)	0(16)	78(67)	18	

From grade	Percents classified into grade				Total observations	
	1	2	3	4		
1	95(85)	0(10)	5(5)	0(0)	20	
2	0(11)	88(73)	6(16)	6(0)	17	
3	0(0)	0(11)	100(89)	0(0)	18	(c)
4	11(6)	6(0)	17(16)	67(78)	18	

Attenuation Measurement

A pulse transmission attenuation method is described by Goss et al. (1979). With minor modifications, the same procedure was used here. Physiological saline was again used as the coupling medium to the tissue specimen. The receiver was axially aligned to the transmitter. Measurement of the sound attenuation was performed as follows. With a Plexiglas sample positioned in the ultrasonic path, the gain of the receiver was adjusted to give an integration value of predetermined amplitude. Then, with the tissue specimen in place, the new integrator value was noted. This attenuation measurement was repeated for several tissue samples of varying fat content. The results indicate attenuation measurement is not suitable for detection of intramuscular fat (Table 9).

Lipid Extraction

Chemical lipid extraction was completed on a Goldfisch apparatus according to A.O.A.C. procedures. The percent fat extracted, recorded as a moisture free basis (MFB), is given for the individual cores of meat in Table 10. These data are divided into four columns, representing the four grades of beef. The criteria used for this division are primarily based on visual grading as given by a qualified grader. Included in this partitioning is the occurrence of natural breaks in the listing of the percentage of fat which could be interpreted as a category separation.

As each core is individually analyzed, it is possible that cores from the same sample are divided in different categories. Fortunately, three cores never ended up in more than two categories. In those muscle

Table 9. Total sample attenuation measurement. Values shown are comparative integration values using a pulsed ultrasonic transmission technique. All values are normalized to a Plexiglas standard. The results indicate attenuation measurement is not a suitable method for detection of intramuscular fat

Tissue sample	Grade	Plexiglas standardization value 1.00	0.70	0.50
244	Prime	.84	.46	.27
250	Prime	.84	.46	.28
254	Prime	.84	.46	.28
300	Prime	.84	.44	.25
306	Prime	.84	.46	.29
310	Prime	.84	.46	.25
328	Prime	.84	.43	.25
334	Prime	.84	.47	.25
338	Prime	.84	.43	.28
342	Prime	.84	.45	.28
348	Prime	.84	.49	.28
352	Prime	.84	.46	.28
230	Choice	.82	.46	.28
236	Choice	.84	.46	.27
240	Choice	.78	.42	.26
286	Choice	.83	.43	.26
292	Choice	.84	.47	.27
296	Choice	.84	.43	.26
314	Choice	.84	.45	.28
320	Choice	.84	.43	.26
324	Choice	.84	.44	.27
216	Good	.84	.46	.29
222	Good	.84	.46	.29
226	Good	.84	.46	.28
258	Good	.84	.42	.27
264	Good	.84	.46	.28
268	Good	.84	.44	.28
272	Good	.84	.45	.28
278	Good	.84	.48	.28
282	Good	.84	.47	.27
200	Standard	.82	.46	.26
205	Standard	.81	.46	.28
210	Standard	.84	.46	.29
356	Standard	.84	.47	.28
362	Standard	.84	.45	.28
366	Standard	.84	.48	.30

Table 10. Fat extraction values of each muscle core sample giving the predetermined visual grade and that grade assigned as a result of this investigation. Codes indicated are: 1 = prime, 2 = choice, 3 = good, 4 = standard, a = both matching core samples in next lower grade, b = both matching core samples two grades lower and c = both matching core samples in next higher grade. Agreement is quite good between the two methods. MFB = moisture free basis

Assigned investigation grade of each meat core											
Prime			Choice			Good			Standard		
% fat (MFB)	Subjective grade		% fat (MFB)	Subjective grade		% fat (MFB)	Subjective grade		% fat (MFB)	Subjective grade	
46.3	1		31.8	2		18.9	3		12.0	4+	
46.1	1		31.5	1	c	18.8	2		11.3	4	
45.3	1		30.1	2-		18.7	3		10.7	4	
43.8	1		29.6	2	c	18.6	2	c	10.4	4	c
43.1	1		28.0	2		18.0	4		10.3	4	
39.7	1		27.5	2-		18.0	3+		10.0	3-	
38.9	2	a	27.1	2		16.5	3+		10.0	4	
38.2	1		25.6	3		16.5	3+		9.8	4	
38.1	2		24.3	2		16.3	3		9.7	3-	
37.3	3+	b	23.7	3		16.1	3+		9.7	4	
37.1	1		23.2	1		15.9	2		9.5	4	
37.0	1		22.8	2	a	15.8	3	c	8.1	4+	
36.3	1		22.8	3	a	15.5	4		7.5	4	
35.1	1		22.5	2		14.8	3-	a	6.8	4	
34.3	1		22.5	2		13.8	3		6.5	4	
34.3	1		22.5	1		13.2	4+	a	6.1	4	
34.1	1		21.0	2-					5.6	4	
33.6	1		20.3	3+	a						
33.0	1		20.0	2							
32.6	2										
32.0	1	a									

samples with one core in a category different from the matching pair, the entire muscle was judged as belonging to the grade in which the pair of extraction values was located. Two reasons can account for sampling error, causing the third core to be graded differently. That section of the muscle from which the core is removed may have a slightly different fat content, or the aliquot of the core which was chemically extracted may not be representative of the entire tissue.

The codes associated with several of the lipid values indicate in which category the matching pair of samples is located. A minus or plus following the predetermined grade indicates that muscle as slightly higher or lower than the average within that grade.

CONCLUSIONS

Three conclusions are drawn from this investigation of beef grading by ultrasound. First, a double transducer system is better able to predict the intramuscular fat distribution than is a single transducer system. There is one qualification in regard to this statement. The double angle scanning technique is valid only when orientation accuracy is maintained. This means that the beam orientation must be consistent from sample to sample. A beam error of 20° will adversely affect the predicted accuracy. In those double-angle analyses where orientation aspects were maintained and individually analyzed, the predicted accuracy was acceptable. When the data from all orientations were combined, the accuracy fell drastically.

If consistent orientation (double-angle scan) proves to be difficult in vivo, the prediction accuracy of a single transducer system may be sufficient. With a single transducer, the transmitter and receiver angle is fixed. Therefore, ultrasonic path length differences of various orientation angles do not affect the results. Consistency of orientation from sample to sample is not critical. It is important that the ultrasonic inspection window be large enough to give a representative sample. As long as this holds true, a single transducer system may produce acceptable results. In anticipation of the electrical and mechanical restrictions that would be attributed to a double transducer system, the tradeoff to a single transducer system with somewhat less accuracy may be a necessity.

Both the results of the double-angle scan and the single transducer analysis point to the second conclusion. There is an orientation aspect with respect to the intramuscular fat deposits. This may best be described as a somewhat irregularly spaced stack of coins. The difference being that the coins would not necessarily be directly under one another. Offsets in all directions would occur.

If the marbling was not specifically oriented, then by combining all three rotational aspects of double-angle scanning, the predicted accuracy would not be reduced as was observed in this investigation. With no particular orientation, path length differences would be random and no mathematical distinction would be observed. The evidence pertaining to the single transducer is not mathematical since beam angle inconsistencies do not affect results. The evidence is in the observation of the ultrasonic signature itself. Through a rotation of 90°, the backscatter pressure amplitude falls drastically as the ultrasonic beam begins to parallel the fat deposits.

The third conclusion to be drawn from this investigation is that ultrasonic grading could not be accomplished in a cold locker as is visually done today. The reason for grading cold carcasses is so that the contrast of fat to lean is more readily observed. Carcasses graded cold typically grade a little higher due to this contrast. If ultrasonic grading could be consistently and accurately accomplished immediately following slaughter, this cold processing delay might be eliminated. The result is a more efficient operation.

One observation from this investigation is noted. The fact that the good grade of marbling is the most reliably predicted may be encouraging. The greatest interest in beef production is in being able to distinguish the good grade from the choice grade. By testing ultrasonically for only the good grade, this dilemma could be solved. Visual conformation would indicate the animal as not being of either grading extreme.

An explanation as to why this particular grade is easier to ultrasonically grade becomes evident upon close examination of the illustrated degrees of marbling. The trend seems to be toward more even distribution as marbling decreases. Therefore, the marbling may be more acceptable for ultrasonic averaging. Also, as marbling decreases the muscle becomes more uniform. Less ultrasonic signal attenuation is the result. The standard grade may be less predictable for two reasons. There may be too little information in the fat that is available ultrasonically, or the ultrasonic window used in this experiment may not have been wide enough to incorporate a representative sample of this particular grade.

It is not known whether the 5 or 10 variables (harmonics) used in this investigation contain sufficient information to ultimately be successful. The indication here is that it may be enough information. Until more parameters with larger numbers of observations are completed, there is no way to know for sure.

Total sample attenuation measurements were not successful for reasons already discussed (i.e., no protein degradation).

Recommendations for Future Research

In light of the mechanics needed to conduct single-frequency Bragg scattering tests, most likely progress would be made using a swept frequency and a single angle. By varying the frequency (usually 1.0 to 5.0 mHz) and using a fixed angle, the effective angle will change, but there is no mechanical involvement. Electronics would take the place of mechanics. The simplified geometrical aspects would result in less potential for error.

The research conducted here attempted to classify all grades of beef. It is likely that by concentrating on only one grade, higher accuracy could be achieved. Gramiak et al. (1976) indicate that a few scatterers may be more predictive than many scatterers. It may be that the ultrasonic time gate used here was too long for accurate prediction of the grades choice or prime. If only one grade was examined, the window size could become the next variable. An optimum window size for any given grade would result. It is possible this same function could be achieved in two other fashions: 1) change the beam width or 2) focus the beam.

One final recommendation would be to change the inspection location. By examining a different muscle, greater structural regularity of intramuscular fat may be found.

LITERATURE CITED

A. O. A. C. 1980. Official methods of analysis. 13th edition. Association of Official Analytical Chemists, Washington, D. C.

Anderson, T. W. 1958. An introduction to multivariate statistical analysis. John Wiley & Sons, Inc., New York. 374 pp.

Armitage, P. 1971. Statistical methods in medical research. Blackwell Scientific Publications, Oxford, England. 504 pp.

Bamber, J.C., M. J. Fry, C. R. Hill and F. Dunn. 1977. Ultrasonic attenuation and backscattering by mammalian organs as a function of time after excision. Ultrasound Med. Biol. 3:15-20.

Bartrum, R. J., Jr., and H. C. Crow. 1977. Gray-scale ultrasound: A manual for physicians and technical personnel. W. B. Saunders Company, Philadelphia. 216 pp.

Chivers, R.C., and C. R. Hill. 1975. A spectral approach to ultrasonic scattering from human tissues: Methods, objectives and backscattering measurements. Phys. Med. Biol. 20(5):799-815.

Dunn, F., P. E. Edmonds and W. J. Fry. 1969. Absorption and dispersion of ultrasound in biological media. Pages 205-332 in H. P. Schwan, ed. Biological engineering. McGraw Hill, New York.

Fink, D. 1979. An investigation of a method for detecting blood flow in aorto-coronary bypass grafts using ultrasound. Unpublished M.S. thesis. Library, Iowa State University, Ames, Iowa.

Firestone, F. A. 1945. The supersonic reflectoscope for interior inspection. Metal Prog. 48:505-512.

Freese, M., and M. A. K. Hamid. 1974. Lipid content determination in whole fish using ultrasonic pulse backscatter. Pages 69-76 in J. deKlerk and B. McAvoy, eds. Ultrasonics symposium proceedings. Institute of Electrical and Electronics Engineers, Inc., New York.

Freese, M., and E. A. Lyons. 1979. Dependence of ultrasound backscatter from human liver tissue on frequency and protein/lipid composition. National Bureau of Standards Special Publication 525:157-163.

French, L.A., J. J. Wild and D. Neal. 1950. Detection of cerebral tumors by ultrasonic pulses. Cancer 3:705-708.

Gammel, P. M. 1981. Beef grading by ultrasound. JPL Invention Report 14812. Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California.

Gibbs, J. 1981. Windowing boosts performance of dynamic signal analyzers. Electronic Design News 26(15):109-113.

Goldberg, B. B., M. N. Kotter, M. C. Ziskin and R. D. Waxham. 1975. Diagnostic uses of ultrasound. Grune and Stratton, New York. 468 pp.

Goss, S. A., R. L. Johnston, V. Maynard, L. Nider, L. A. Frizzell, W. D. O'Brien, Jr. and F. Dunn. 1979. Elements of tissue characterization. Part II. Ultrasonic propagation parameter measurements. National Bureau of Standards Special Publication 525:43-51.

Gramiak, R., L. P. Hunter, P. P. K. Lee, R. M. Lerner, E. Schenk and R.C. Wagg. 1976. Diffraction characterization of tissue using ultrasound. Pages 60-63 in J. deKlerk and B. McAvoy, eds. Ultrasonics symposium proceedings. Institute of Electrical and Electronics Engineers, Inc., New York.

Hill, C. R. 1976. Frequency and angular dependence of ultrasonic scattering from tissue. National Bureau of Standards Special Publication 453:197-206.

Howry, D. H., and W. R. Bliss. 1952. Ultrasonic visualization of soft tissue structures of the body. J. Lab. Clin. Med. 40:579-592.

Huggins, R. W., and J. V. Phelps. 1976. Bragg diffraction scanner for ultrasonic tissue characterisation *in vivo*. Ultrasound Med. Biol. 2:271-277.

Johnston, R. L., S. A. Goss, V. Maynard, J. K. Brady, L. A. Frizzell, W. D. O'Brien, Jr. and F. Dunn. 1979. Elements of tissue characterization. Part I. Ultrasonic propagation properties. National Bureau of Standards Special Publication 525:19-27.

Lele, P. P., A. B. Mansfield, A. I. Murphy, J. Namery and N. Senapati. 1976. Tissue characterization by ultrasonic frequency-dependent attenuation and scattering. National Bureau of Standards Special Publication 453:167-196.

Lerski, R. A., M. J. Smith, P. Morley, E. Barnett, P. R. Mills, G. Watkinson and R. N. M. MacSween. 1981. Discriminant analysis of ultrasonic texture data in diffuse alcoholic liver disease. Ultrasonic Imag 3(2):164-172.

Ludwig, J., and F. Struthers. 1950. Detecting gall-stones with ultrasonic echoes. Electronics 23(2):172-178.

McDicken, W. N. 1976. Diagnostic ultrasonics. Crosby Lockwood Staples, London. 320 pp.

Nicholas, D. 1976. The application of accoustic scattering parameters to the characterisation of human soft tissues. Pages 64-69 in J. deKlerk and B. McAvoy, eds. Ultrasonics symposium proceedings. Institute of Electrical and Electronics Engineers, Inc., New York.

Nicholas, D., and C. R. Hill. 1975. Tissue characterization by an acoustic Bragg scattering process. Pages 269-272 in L. J. I. Browne, ed. Ultrasonics International Conference Proceedings. I. P. C. Science and Technology Press, London.

Pauly, H., and H. P. Schwan. 1971. Mechanism of absorption of ultrasound in liver tissue. J. Acoust. Soc. Am. 50:692-699.

Rose, J. L., and B. B. Goldberg. 1979. Basic physics in diagnostic ultrasound. John Wiley & Sons, Inc., New York. 340 pp.

Sagar, K. B., T. H. Rhyne, G. S. Myers and R. S. Lees. 1978. Characterization of normal and abnormal pulmonary surface by reflected ultrasound. Chest 74(1):29-33.

Shung, K. K., and J. M. Reid. 1977. Ultrasonic scattering from tissues. Pages 230-233 in J. deKlerk and B. McAvoy, eds. Ultrasonics symposium proceedings. Institute of Electrical and Electronics Engineers, Inc., New York.

Stouffer, J. R., and R. G. Westervelt. 1977. A review of ultrasonic applications in animal science: Reverberations in echocardiograms. J. Clin. Ultrasound 5(2):124-128.

Waag, R. C., P. K. Lee, R. M. Lerner, L. P. Hunter, R. Gramiak and E. A. Schenk. 1979. Angle scan and frequency-swept ultrasonic scattering characterization of tissue. National Bureau of Standards Special Publication 525:143-156.

Wells, P. N. T. 1969. Physical principles of ultrasonic diagnosis. Academic Press, New York. 635 pp.

Wild, J. J. 1950. The use of ultrasonic pulses for the measurement of biological tissues and detection of tissue density changes. Surgery, St. Louis 27:183-188.

Wild, J. J., and J. M Ried. 1952. Further pilot echographic studies on the histologic structure of the living intact human breast. Am. J. Pathol. 28:839-861.

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